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The Metabolism of 3,4-Benzpyrene in Mice and Rats

I. The Isolation of a Hydroxy and a Quinone Derivative, and a Consideration of Their Biological Significance*†

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From previous metabolic studies, it is known that conversion of carcinogenic hydrocarbons into hydroxy derivatives can occur in the animal body. In the case of 3,4-benzpyrene, a fluorescent substance (BPX), discovered by Peacock and Chalmers in the bile (24) and the feces (14, 10, 11), is considered by Chalmers and Crowfoot (12) to be a monohydroxy derivative, while in the case of 1,2,5,6-dibenzanthracene, a dihydroxy derivative has been isolated from the excreta by several workers (7, 8, 18, 19). The position of the hydroxy group in the benzpyrene derivative was not established by previous workers. The dibenzanthracene derivative was identified as 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene in mice and rats (9, 19), but has not yet been identified in rabbits (7, 8, 18, 19). Metabolic derivatives of cholanthrene and 20-methylcholanthrene have so far not been isolated, but the detection of alkali-soluble fluorescent products in the bile of animals injected with these hydrocarbons (15), suggests that a similar metabolic mechanism is probably involved. No metabolic products of carcinogenic hydrocarbons other than hydroxy derivatives have so far been described.

The present work deals with a reinvestigation, on a more extensive scale, of the problem of the metabolism of benzpyrene, made possible by improving the conditions determining the production of the metabolic products, and by simplifying the method of their extraction. The identification of these products is considered in a separate communication (4).

FACTORS AFFECTING THE EXCRETION OF METABOLIC PRODUCTS OF 3,4-BENZPYRENE

In view of the small amount of BPX produced by the animal under previous conditions, a reinvestigation of the factors influencing production was necessary.

In the case of intravenous injection of a colloidal solution of benzpyrene, the low yield of BPX in the excreta is obviously due to the small dose which can be introduced by the intravenous route. In the case of subcutaneous injection, by which large amounts of benzpyrene in oil can be introduced into the animal, the low yield of BPX is attributable to the very slow rate of metabolic change. This was demonstrated by the present authors in a previous communication (5), when it was found that the rate of disappearance of benzpyrene is about 15 times greater when injected intraperitoneally than when injected subcutaneously, and that this disparity is due primarily to a difference in the rate of transfer of the unchanged benzpyrene from the site of injection to the site of metabolic transformation and elimination. Since the increase in the rate of transfer, in the case of intraperitoneal injection, is not accompanied by any significant accumulation elsewhere in the body, or by any significant increase in excretion, of the unchanged benzpyrene, it follows that the tissues in which the metabolic change occurs are capable of coping with all the benzpyrene brought to them.

From this, it was to be expected that the feces of animals injected *intraperitoneally* with *large* doses of benzyprene, would serve as rich sources of metabolic products of this hydrocarbon. This was in fact found to be the case.

The amounts injected were about 40 mgm. of benzpyrene in 2 ml. of sesame oil in rats, and about 10 mgm. in 0.5 ml. of sesame oil in mice. (Of the two, the mouse is the more efficient in metabolizing benzpyrene, probably owing to the larger surface area of its peritoneal cavity in relation to its body size.) The animals were kept in darkened cages, and the feces collected at 3 to 5 day intervals for 10 to 15 days. The feces were subsequently ground to a fine powder and kept in a desiccator over H_2SO_4.

* A preliminary note on this work has been published (6).

† Because of the difficulties of international communication the proof sent to the authors did not come back.

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Extraction and Isolation of Metabolic Products of 3,4-Benzpyrene from the Feces

Extraction of the feces with boiling ethanol under continual reflux, as recommended by Chalmers (11), was found to be wasteful, owing to considerable decomposition of the labile metabolic products. The subsequent procedure recommended for the purification of BPX, involving evaporation in N_2, solution in NaOH, repeated chilling, and removal of insoluble matter, followed by acidification and transference to benzene, before chromatographic adsorption, was found to be both wasteful and unduly complicated for large scale work.

The method described below, though theoretically less efficient for quantitative extraction, yet in the end led to reasonably good yields as destruction of the labile products was greatly minimized. In view of the marked photosensitivity of BPX, the various stages of purification were carried out in subdued light or, whenever possible, in total darkness.

The powdered feces were packed into a tall glass column, having a constriction below plugged with a wedge of cotton wool, and cold benzene was allowed to percolate slowly for long periods, the progress of extraction being judged by the intensity of the fluorescence of the extract. The benzene extract was then passed through short, wide columns of aluminium oxide [1] for chromatographic separation. A brownish grey zone was adsorbed at the top; this was followed by an intermediate zone (ill defined in daylight at this stage, but easily recognizable in ultraviolet light by its strong yellow fluorescence); and below this was a deep red zone, slowly travelling down the column. On developing the column with chloroform, the red zone was eluted; while the intermediate zone containing the BPX travelled down slowly and became distinct from the upper zone as a light greenish yellow zone with intense yellow fluorescence in ultraviolet light. This zone was cut and eluted with methanol containing about 1 per cent ammonia. The methanolic extract was evaporated *in vacuo,* yielding a yellow material that exhibited an intense green fluorescence in alkaline solutions and a strong blue fluorescence when dissolved in neutral organic solvents.

Further purification of this material by repeated chromatography proved unsatisfactory owing to serious losses, partly through incomplete elution from the alumina but mainly through decomposition during manipulation. However, on sublimation in high vacuum, by use of the micro-still of Strain and Allen (28) and a mercury vapor pump, a pale yellow material containing needle-shaped crystals was obtained, which appeared identical with the "crystalline BPX" of Chalmers and Crowfoot (12).

The red fraction, eluted from the alumina column with chloroform, was further investigated. Since this fraction was not found in extracts of normal feces it seemed reasonable to suppose that it, too, might be a derivative of benzpyrene.

For its purification, the chloroform eluate was evaporated to dryness, taken up in light petroleum, in which it was only sparingly soluble, and passed through another column of alumina, on which it was strongly adsorbed. The column was washed with large quantities of light petroleum, which eluted any unchanged benzpyrene present as well as large amounts of a colorless crystallizable material, probably a sterol. The red zone was then cut and eluted with chloroform. The procedure was repeated, and on evaporation of the final chloroform eluate, rosettes of deep brownish red crystals separated out. This material was further purified by high vacuum sublimation and recrystallization from glacial acetic acid.

This crystalline red product was found to be readily soluble in chloroform and hot glacial acetic acid, moderately soluble in benzene, ethanol, and acetone, sparingly soluble in light petroleum, and insoluble in water at any pH.

In solution it is yellow in color with no discernible fluorescence. It is not readily destroyed by irradiation with ultraviolet light and in many other ways is more stable than BPX. With concentrated H_2SO_4 it gives an olive green coloration. In all these respects its behavior is similar to that of the 3,4-benzpyrene-5,8-quinone, as synthesized by Vollmann and his associates (30), and this was further supported by the fact that a mixture of the two could not be separated by chromatography.

Evidence Concerning the Extent of Metabolic Transformation

In view of the instability of BPX, quantitative estimations of its concentration in the excreta are unreliable. Approximate data could still be of value, however, if they could indicate whether the excretion of BPX could account for the greater part of the benzpyrene that disappears from the body, or whether, as might be inferred from the observations of Chalmers and Crowfoot (12), it represents only a small fraction of the loss.

Such approximate values were obtained by matching the intensities of fluorescence of the crude benzene extracts of mouse feces, in various dilutions, with known standards. It was found that feces collected during the first 3 days after intraperitoneal injection of 10 mgm. of benzpyrene contained about 1 to 1.5 mgm. of fluorescent material. Since about 6 mgm. of

[1] "Aluminium oxide for adsorption purposes," British Drug Houses, Ltd.

the 10 mgm. injected had disappeared from the body during these 3 days, while less than 1 per cent of this was excreted as unchanged benzpyrene (5), it may be concluded that one-sixth to one-fourth of the amount lost could be accounted for as fluorescent metabolic products in the feces. The amounts of fluorescent metabolic products excreted in the urine were not estimated.

Carcinogenicity of Metabolic Products of 3,4-Benzpyrene

The small amounts of metabolic products available, and their instability on keeping, prevented large scale experiments on carcinogenesis by skin painting. Tests by subcutaneous injection seemed more feasible, and these were therefore undertaken on groups of 10 mice, both with respect to the metabolic hydroxybenzpyrene (BPX) and the quinone derivative obtained from the feces.

Crude preparations of these products were used, dissolved or suspended in sesame oil. Single injections of 0.5 ml. were given subcutaneously in the sacral region. From approximate estimations of the crude materials used, the amounts of pure BPX and quinone respectively appeared to be of the order of 2 mgm. per mouse.

The animals tolerated the treatment well, most of them remaining alive after 7 months. Some were killed for autopsy and chemical examination between the 7th and 10th months, while 3 of the BPX series and 4 of the quinone series were still alive after 10 months, when they were killed. There was evidence of persistence of the injected substance *in situ* in both series, though in the animals injected with the quinone, a changed product—salmon pink in color, and strongly adsorbed on alumina—was also present. Its nature has not yet been determined.

In the quinone series, none of the animals developed any tumors. In the BPX series, one mouse, killed after 10 months, had a small nodule attached to the thin capsule around the injected material, which proved histologically to be a spindle cell sarcoma; another of this series had a typical mammary carcinoma away from the site of injection (left axilla), which was presumed to have developed spontaneously; the other mice of this series were free from tumors.

In contrast to these results, 3 groups of 20 mice, injected subcutaneously with 2.0, 0.2, and 0.02 mgm. of benzpyrene in sesame oil, yielded 90, 40, and 15 per cent of tumors respectively under comparable conditions.

DISCUSSION

Three separate problems are involved in the elucidation of the biological activities of a carcinogenic hydrocarbon such as 3,4-benzpyrene: (a) the rate of disappearance of the unchanged hydrocarbon; (b) the nature of the substances into which it is transformed in the body; and (c) the distinction between those transformed substances which may be concerned with the biological activities of the parent hydrocarbon (carcinogenesis, inhibition of tumor growth, estrogenic action, etc.), and those which are not.

The first of these problems has already been considered by the present authors in a previous publication (5), from which three significant conclusions may be drawn: First, the limiting factor that determines the rate of disappearance of benzpyrene from the mouse is the rate of diffusion of the unchanged hydrocarbon from the site of injection to the blood, and not the rate of its metabolic transformation or excretion after transference *via* the blood stream. Second, more than 99 per cent of the benzpyrene lost from the animal must be accounted for by conversion into metabolic products—a fact already observed by Chalmers and Kirby (13) with regard to rats. Third, persistence of unchanged hydrocarbon *in situ* seems to be necessary for local carcinogenic action.

Reference has already been made to the earlier work on the isolation of a metabolic product of benzpyrene (BPX) by Peacock, Chalmers, and Crowfoot (24, 14, 10, 11, 12) and to the conclusion that it was in all probability a monohydroxy derivative. The present investigation has led, in addition, to the isolation of a second derivative of benzpyrene, possessing properties similar to synthetic 3,4-benzpyrene-5,8-quinone. The identification of the red product with 3,4-benzpyrene-5,8-quinone, and the evidence for concluding that the hydroxy group in BPX occupies the 8- position, are described in a separate communication (4).

Since 8-hydroxy-3,4-benzpyrene is very labile, quantitative data concerning its excretion cannot be accurate. Approximate comparisons of the intensities of fluorescence of crude extracts of feces with those of known standards are useful, however, in that the values obtained represent minimal values. Such comparisons have shown that one-sixth to one-fourth of the benzpyrene lost from the body can be accounted for in the form of fluorescent material. Since the part excreted in the urine is not included in this, and the values for feces are too low, owing to losses through decomposition before and during extraction, it is probable that the metabolized fluorescent products in the excreta must account for a considerable fraction of the benzpyrene that had disappeared from the body, and that the very small yields of the purified fluorescent material bear little relation to the amount actually excreted. That the fluorescent products do not account for the whole of the lost benzpy-

rene is evident, however, from the presence also of a nonfluorescent benzpyrene-quinone in the feces.

Unlike the hydroxy derivative (BPX), the red quinone possesses no specific fluorescence, and its detection in the body is therefore rendered difficult. Moreover, its presence in purified extracts of tissues or feces does not necessarily prove that it is a product of body metabolism, since the conversion of BPX to the quinone may have occurred by auto-oxidation. From the evidence available, it is not possible to state whether the quinone is or is not produced metabolically.

With the isolation and identification of two derivatives of benzpyrene from the feces, and the knowledge that the two probably account for the greater part of the benzpyrene disappearing from the body, the third of the problems mentioned above becomes a matter of considerable interest; namely, whether these derivatives play any part in the biological activities of the parent hydrocarbon.

In this connection, the striking diminution in carcinogenicity of these derivatives of benzpyrene, in relation to the parent hydrocarbon, may be compared with that of other hydroxy and quinone derivatives of carcinogenic hydrocarbons described in the literature.

Of the synthetic hydroxy derivatives of 3,4-benzpyrene tested for carcinogenesis, the 4′- (1, 2, 27), 2- (20), and 5- (26) were negative, and the 6- (20) slightly positive; of the hydroxy derivatives of 1,2-benzanthracene, the 8- (20) and 10- (26, 27) were negative and the 3- (26, 27) reported as both negative and positive; while 3-hydroxy-10-methyl-1,2-benzanthracene (27) was also negative; and of the hydroxy derivatives of 20-methylcholanthrene, negative results were obtained with the 2- (27) and 3- (2, 27) and positive results with the 15- (27). Of particular interest in this connection is the fact that 4′, 8′-dihydroxy-1,2,5,6-dibenzanthracene (the metabolic derivative of the parent hydrocarbon) is also noncarcinogenic (8, 20).

Similarly, in the case of the quinone derivatives of carcinogenic hydrocarbons, 1,2-benzanthraquinone (27), 1,2,5,6-dibenzanthraquinone, and 3,4,9,10-dibenzpyrene-5,8-quinone (22) were all found to be noncarcinogenic.

The fact that the metabolites of carcinogens, and their synthetic analogues, are considerably less carcinogenic than their parent hydrocarbons confirms the conclusion already reached by indirect evidence (5) that local carcinogenesis depends on persistence of the unchanged hydrocarbon, and not on the presence of its metabolites.

The other biological activities that many of the carcinogenic hydrocarbons possess, namely inhibition of tumor growth (22, 23, 3) and estrogenic action (16, 17, 25), differ in two essential respects from carcinogenesis: (a) The amounts necessary to produce such effects are many hundred times greater than those required for carcinogenesis. (b) The effects are produced at a distance from the site of injection. The possibility that these remote effects of carcinogenic hydrocarbons are due to some of their metabolic products rather than to the parent hydrocarbons themselves must therefore be seriously considered.

Until now, the metabolic conversion of a carcinogenic hydrocarbon has only been considered as a detoxication mechanism in the *negative* sense of loss of carcinogenic propensities (19, 9). The suggestion now put forward is that the metabolites may also have *positive* action.

As regards inhibition of tumor growth, while some hydroxy and quinone derivatives of hydrocarbons have little or no inhibitory action, *e.g.* 1,2,5,6-dibenzanthraquinone (23), 1,9-benzanthrone (22), anthanthrone, pyranthrone, dibenzanthrone, *iso*-dibenzanthrone, and allomesonaphthodianthrone (3), definite inhibitory effects have been obtained with 4′-hydroxy-3,4-benzpyrene, 9,10-dihydroxy-9,10-di-N-propyl-9,10-dihydro-1,2,5,6-dibenzanthracene (22), the two dibenzpyrene-quinones, 4-methyl- and 4-aldehyde-benzanthrone, and 1,2,5,6-dibenzfluorenone (3). Thus the positions of the hydroxy and quinone groups may strongly influence inhibitory action. However, the fact that several hydroxy and quinone derivatives of carcinogenic hydrocarbons possess strong inhibitory action, while none of those so far tested possess more than slight carcinogenic action, seems significant. The fact that the experimental conditions which most favor inhibitory action (*i.e.* injection by the intraperitoneal route) are also those most effective in eliciting metabolic products, also supports the view that preliminary conversion of a carcinogen into its metabolites may play a necessary part in the inhibitory effect of the parent hydrocarbon.

Finally, in discussing the influence which the position of the hydroxy or quinone groups may have in determining inhibitory action, attention must also be drawn to the fact that the types of metabolic products may be different in different species. It has already been shown (7, 8, 18, 19, 9) that in the case of 1,2,5,6-dibenzanthracene the hydroxy derivative found in the rabbit is different from that excreted by mice and rats. Preliminary investigations by the present authors have shown that, contrary to the statement of Chalmers and Crowfoot (12), the metabolism of 3,4-benzpyrene is also different in the rabbit from that in the rat or mouse.

The available data for estrogenic activity of hydrocarbons (16, 17, 25) and their derivatives are too scanty for conclusions to be reached on the subject. It may be significant that so many of the potent estrogens are hydroxy derivatives, and that those synthetic com-

pounds that are not, are metabolized in the body into phenolic derivatives that are more active than their parent compounds (29, 21). But in order to decide whether the estrogenic effect of a carcinogen is due to its preliminary conversion into a hydroxy compound, the known metabolites would themselves have to be tested for such action.

It may be concluded, therefore, that whereas the carcinogenic action of a polycyclic hydrocarbon does not depend on conversion into hydroxy or quinone derivatives, the arguments in favor of these metabolites playing an essential part in some of the other biological activities that a carcinogen may possess (inhibitory action on tumor growth and estrogenic action) appear to be sufficiently plausible to warrant further investigation.

SUMMARY

Injection of 3,4-benzpyrene by the intraperitoneal route in mice or rats was found to be most favorable in providing high yields of metabolic products in the feces.

By a simplified method of extraction two products were isolated from the feces: a fluorescent phenolic derivative, identical with BPX of Peacock, Chalmers, and Crowfoot, and another product, not previously described, with properties similar to synthetic 3,4-benzpyrene-5,8-quinone.

From approximate estimations it seems probable that the greater part of the benzpyrene lost from the body appears in the excreta in the form of these two isolated derivatives.

The quinone was found to be noncarcinogenic after 10 months, following subcutaneous injection of about 2 mgm. in sesame oil. Similar injections of the metabolic 8-hydroxy product (BPX) yielded a sarcoma in 1 mouse of 10.

The significance of the conversion of 3,4-benzpyrene into these two derivatives, in relation to the biological activities of the parent hydrocarbon, is discussed. While these derivatives probably play no part in the carcinogenic mechanism of the parent hydrocarbon, the arguments in favor of their being concerned with other biological activities (*e.g.* inhibition of tumor growth and estrogenic action) are suggestive, and call for further investigation.

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The Metabolism of 3,4-Benzpyrene in Mice and Rats

II. The Identification of the Isolated Products as 8-Hydroxy-3,4-Benzpyrene and 3,4-Benzpyrene-5,8-Quinone *

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The present work deals with the identification of the hydroxy and quinone derivatives of 3,4-benzpyrene, extracted from mouse and rat feces, as described in the preceding paper (1). In view of the labile nature of the hydroxy compound, BPX, and for other technical reasons, direct comparisons between these products and synthetic compounds were not actively pursued. Instead, the products from the feces and some synthetic derivatives of benzpyrene were converted into methoxy and other derivatives, and comparisons were made of their crystallographic character and their fluorescence and absorption spectra.

Synthetic Conversion of the Isolated Products into Other Derivatives

Hydroxy derivatives.—Attempts were made to convert the red quinone derivative isolated from the feces (1) into a hydroxy derivative, by reduction with Zn and H_2SO_4. A greenish yellow fluorescent product was obtained, which was strongly adsorbed on alumina. This could not be isolated, however, owing to its great instability in air, especially in the alkaline state, when it was almost instantaneously reconverted into the red compound. The same occurred on reduction of the mixture of synthetic 5,8- and 5,10-quinones, prepared from benzpyrene by the method of Vollmann and his collaborators (6). Catalytic hydrogenation (with Adams' catalyst and H_2 at atmospheric pressure) yielded similar unstable fluorescent products.

These results were compatible with the view that, on reduction, both the red product from the feces and the synthetic quinones were converted into dihydroxy derivatives of benzpyrene.

Acetoxy derivatives.—Reduction of the red product from the feces and of the synthetic quinones in the presence of acetic anhydride and pyridine, yielded the more stable acetoxy compounds, but these also were not sufficiently stable to provide good yields of pure crystalline products with the small amounts of material available. Sufficient amounts of the crude products were obtained, however, for spectrographic analysis of their fluorescence.

Methoxy derivatives.—The production of methoxy derivatives of benzpyrene by methylation of the metabolic hydroxy compound (BPX), and by reductive methylation of the red product from the feces and the synthetic quinones, proved to be the most satisfactory approach in the elucidation of the structures of the substances concerned, the derivatives being fairly stable and yielding readily crystalline products.

For the methylation of BPX, the crude fluorescent fraction from the feces, at the stage after evaporation *in vacuo* of the methanolic extract (1), was dissolved in excess of dimethyl sulfate, and heated with concentrated aqueous NaOH for a short time; then it was well cooled and treated with methanol to facilitate the destruction of the excess of dimethyl sulfate by the alkali present. After further heating to complete the reaction, the mixture was diluted with water and the product transferred to benzene. The benzene extract was washed repeatedly with water, dried with anhydrous Na_2SO_4, and passed through a column of alumina. The methoxy compound passed through into the eluate, while unchanged BPX and other impurities remained adsorbed.

The benzene eluate exhibited an intense blue fluorescence similar to that of BPX but, unlike the latter, the fluorescence remained unchanged on addition of alkali.

The solution was evaporated to dryness and sublimed in high vacuum. The main fraction distilled at about 110° C., and this was recrystallized from amyl acetate. A second fraction, coming over at about 150° C., was present in too small an amount for crystallization.

For reductive methylation of the red product from

* Because of the difficulties of international communication the proof sent to the authors did not come back.

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*** R. S. is indebted to the Nuffield Trust for a personal grant.

the feces, the substance was dissolved in dimethyl sulfate and boiled with concentrated aqueous NaOH and powdered zinc, until the mixture changed to a greenish yellow color, care being taken that sufficient NaOH was always present to maintain an alkaline reaction. The subsequent procedure was the same as in the case of the methylation of BPX, and on sublimation in high vacuum the greater part came over at about 150° C. (From subsequent comparisons it became clear that the second fraction of the methylated BPX, mentioned previously, was identical with the present product, its presence being due to a slow transformation of BPX into the quinone during the process of purification.)

The mixture of 5,8- and 5,10-quinones, obtained synthetically by the oxidation of benzpyrene with chromic acid (6), was treated in the same way as the red product from the feces, and on sublimation in high vacuum of the resulting methoxy compounds, two fractions were obtained—one at about 100° C. and the other at about 150° C. Both were further purified by repeated crystallization. Subsequently small samples of the individual quinones (5,8- and 5,10- respectively) became available,[1] and these were separately converted into the corresponding methoxy compounds. By this means it became possible to establish that the fraction of the mixture coming over at about 150° C. was mainly the 5,8- derivative, and the one at about 100° C. the 5,10- derivative.

Analysis

Samples of the methoxy compounds derived from the methylation of BPX and reductive methylation of the mixture of synthetic quinones, were submitted for quantitative analysis in order to determine the number of methoxy groups present.

Analysis (Strauss and Weiler).—

Methoxybenzpyrene from BPX (major fraction coming over at about 110° C.)

Sample 1 = 9.88 per cent methoxy
Sample 2 = 8.02 " " "

(Monomethoxybenzpyrene requires 11.0 per cent methoxy.)

Methoxybenzpyrene from the mixed synthetic quinones

Sample 1 = 17.1 per cent methoxy
Sample 2 = 18.5 " " "

(Dimethoxybenzpyrene requires 19.9 per cent methoxy.)

[1] The separate samples of 5,8- and 5,10-quinones were kindly supplied to us by Dr. F. L. Warren, of the Chester Beatty Research Institute of the Royal Cancer Hospital. They were prepared by oxidation of benzpyrene with chromic acid, and separated through their acetyl derivatives, according to the method of Vollmann and his collaborators (6).

Fluorescence Spectra

The distinctive fluorescence spectra of benzpyrene and of many of its derivatives can be of considerable help in distinguishing the various compounds, even in the presence of many impurities.

In the present work a semi-quantitative method of determining relative fluorescence intensity distribution in the wave length region 3,800–5,400 Å afforded a rapid and sensitive means of characterizing benzpyrene and its derivatives. A series of photographs of the fluorescence spectrum of the test substance in solution of graded concentration enabled curves of approximate intensity distribution to be drawn. The method provides a much better representation than can be got from a single exposure. Examples of such curves are given in Fig. 1.

The results obtained from a study of the fluorescence spectra of the different products and derivatives in benzene may be summarized as follows:

1. BPX (Fig. 1, a) gave three major bands, the strongest being preceded by some fluorescence around 4,150–4,250 Å. In addition, two bands of variable intensity were observed, one at about 3,900 Å and the other in the region of 5,400 Å. The former may possibly have been due to impurities; the latter (probably extending further towards the long wave length when photographed on plates more sensitive to red) was weak when the BPX was dissolved in a neutral solvent (benzene) and very intense when in the presence of alkali (*e.g.* in benzene-methanol-ammonia mixture). This would account for the change in naked eye fluorescence from blue to green on addition of alkali.

2. The fluorescence bands of methylated BPX were practically identical with those of BPX itself in benzene, except that the band at the long wave length did not increase in intensity on addition of alkali.

3. In the case of the dimethoxybenzpyrenes, obtained by reductive methylation of the synthetic quinones, the bands for the 5,8- were different from those of the 5,10- (Fig. 1, b and c), while those of the mixture appeared as a composite picture of the two (Fig. 1, d).

4. The bands of the methoxy compound obtained by reductive methylation of the red product from the feces (Fig. 1, e) were indistinguishable from those of the synthetic 5,8- compound (Fig. 1, b).

5. The methoxy compound obtained by oxidizing BPX to the quinone followed by reductive methylation, exhibited bands which were similar to (b) and (e) rather than to (d) of Fig. 1. It is clear from this that the oxidation product was a single quinone (5,8-) and not a mixture.

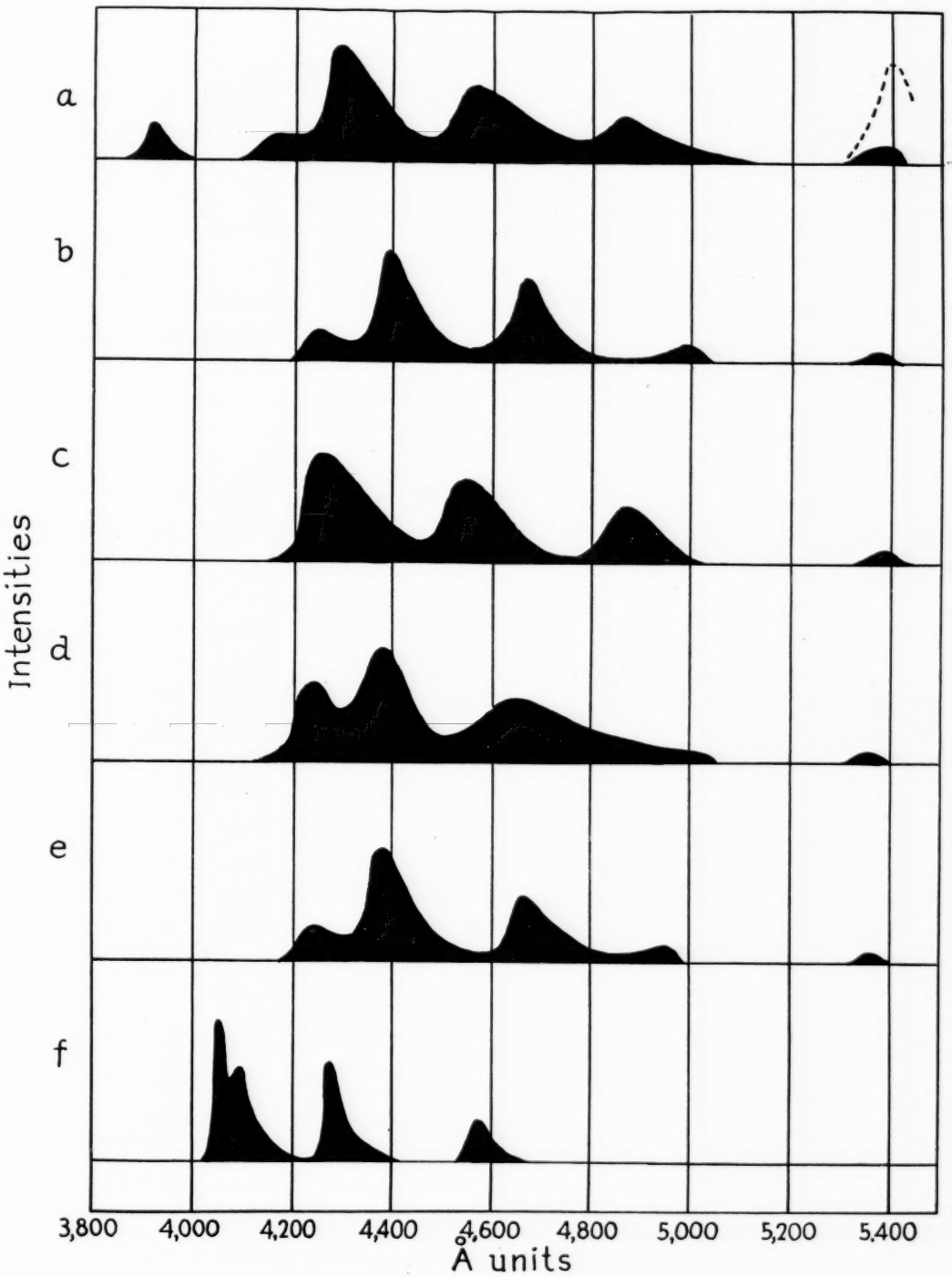


FIG. 1.—Semi-quantitative representations of fluorescence intensity distribution.
a = Metabolic hydroxy derivative (BPX).
b = Synthetic 5,8-dimethoxy-3,4-benzpyrene.
c = Synthetic 5,10-dimethoxy-3,4-benzpyrene.
d = Mixture of synthetic 5,8- and 5,10-dimethoxy-3,4-benzpyrene.
e = Reductive methylation product of red substance from the feces.
f = Benzpyrene.

Absorption Spectra

Preliminary investigations were made with the method previously described by one of the authors (5). This method was found useful for rapidly comparing absorption band systems of a crude mixture with those of purified reference substances. When purified products were ultimately obtained from the crude mixtures, final checking was made by comparing plots of absorption curves obtained with the benzpyrene (BPX) in both ethanol and hexane. In Fig. 2, the effect of methylation of the metabolic hydroxybenzpyrene on the absorption spectrum is shown.

From Figs. 2 and 3 it can be seen that definition of absorption band systems increases from the hydroxy in ethanol, to hydroxy in hexane, to methoxy in hexane. This is an added reason for using the methoxy derivative in hexane as a standard reference. The low solubility of these compounds in hexane has been given as a reason for not using this solvent (4). If, however,

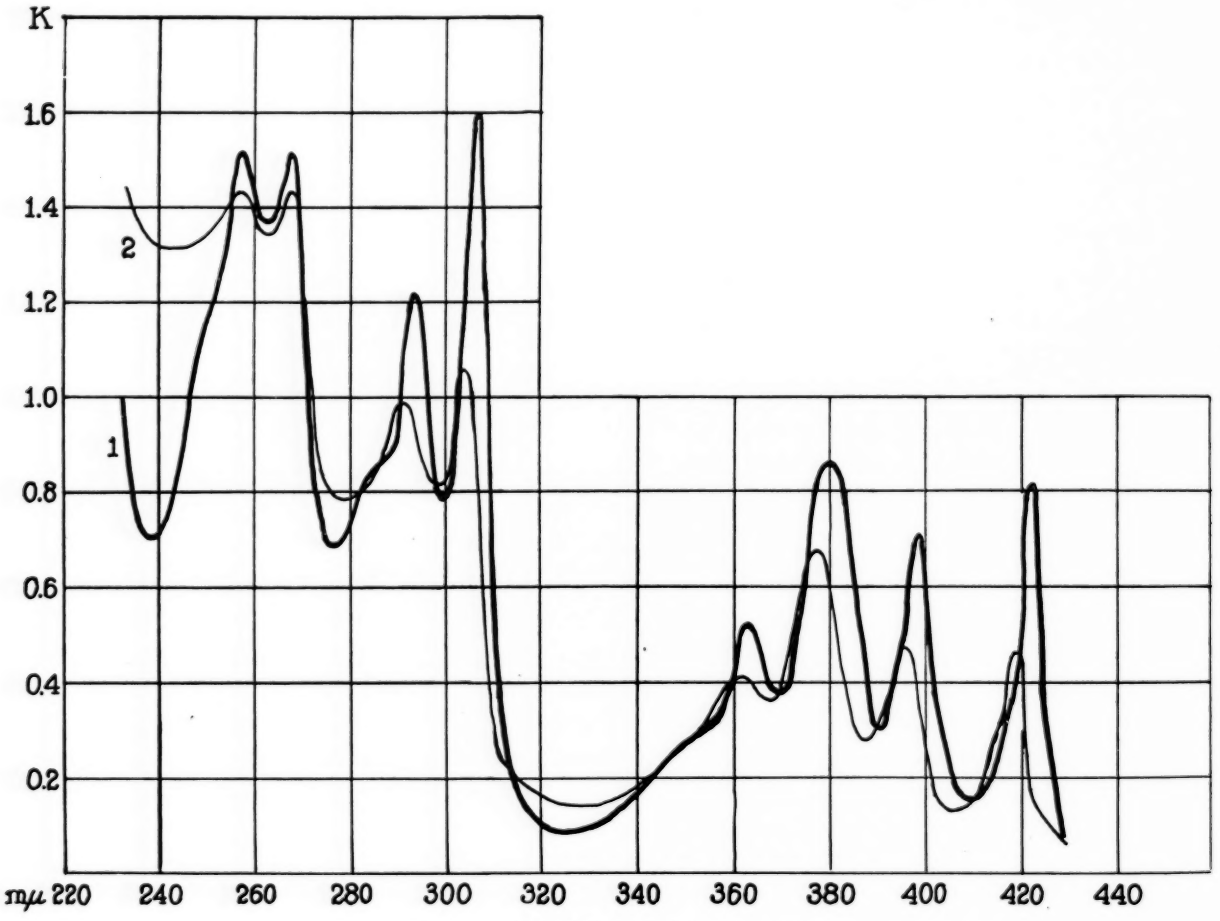


Fig. 2.—Absorption spectra.
1. Methoxy derivative of BPX in hexane.
2. BPX in hexane.

Spekker photometer. The spectrograph was a Hilger A 316 with flat focal plane adaptation. The cells used were 5 cm. long.

Identity of absorption of the methoxy compound derived from the red product from the feces, with that of synthetic 5,8-methoxy-3,4-benzpyrene, is shown in Fig. 4. By contrast, the nonidentity with 5,10-dimethoxy-3,4-benzpyrene is shown in the same figure. In Fig. 3 is reproduced the curve of 5-hydroxy-3,4-benzpyrene in ethanol, transformed from the paper of Fieser and Hershberg (4). This obviously is signally different from that of the metabolic hydroxy-

a long cell is used (5 to 10 cm.) very low dilutions, of the order of 1×10^{-6} molar, may be accurately measured in the case of unsaturated polycyclic hydrocarbons.

Crystallographic Measurements

Crystallographic measurements were made on several preparations of different degrees of purity of crystalline methylated BPX and also of the methoxy derivatives obtained by reductive methylation of both the synthetic quinones and the red product from the feces. In these, three crystallographic species only

could be distinguished: methylated BPX, 5,8-dimethoxy-3,4-benzpyrene, and 5,10-dimethoxy-3,4-benzpyrene (Table I and Fig. 5).

Morphological and Optical Examination

The crystals of 5,10-dimethoxy-3,4-benzpyrene are lath-shaped plates with straight extinction, distinct in form from the crystals of 5,8-dimethoxy-3,4-benzpyrene and methylated BPX. A small proportion of crystals similar to these, and probably to be identified with them, occurred in the preparation of the 5,8- fraction from the synthetic mixture obtained by fractional sublimation.

Methylated BPX and 5,8-dimethoxybenzpyrene crystallize in fine needles, which can be distinguished by morphological and optical examination, as indicated in Table I and Fig. 5. They are, however, so similar that some doubt was felt about their nonidentity until x-ray measurements were carried out. The crystals of the reductive methylation derivative of the red product from the feces were indistinguishable from those of 5,8-dimethoxy-3,4-benzpyrene.

X-Ray Crystallographic Measurements

X-ray measurements were made on: (a) a sample of small good crystals of methylated BPX, and (b) crystals of 5,8-dimethoxy-3,4-benzpyrene isolated from the mixed methylated quinone preparation. Photographs were later obtained from the dimethoxybenzpyrene prepared from the red product of the feces, and these proved to be identical with those of the synthetic 5,8- compound.

The crystal structures of methylated BPX and 5,8-dimethoxybenzpyrene are similar and are of an interesting and complex triclinic variety. The simplest unit cell contains four molecules, but for ease of measurement the structure has been referred to a larger unit, in which the angles between crystal axes

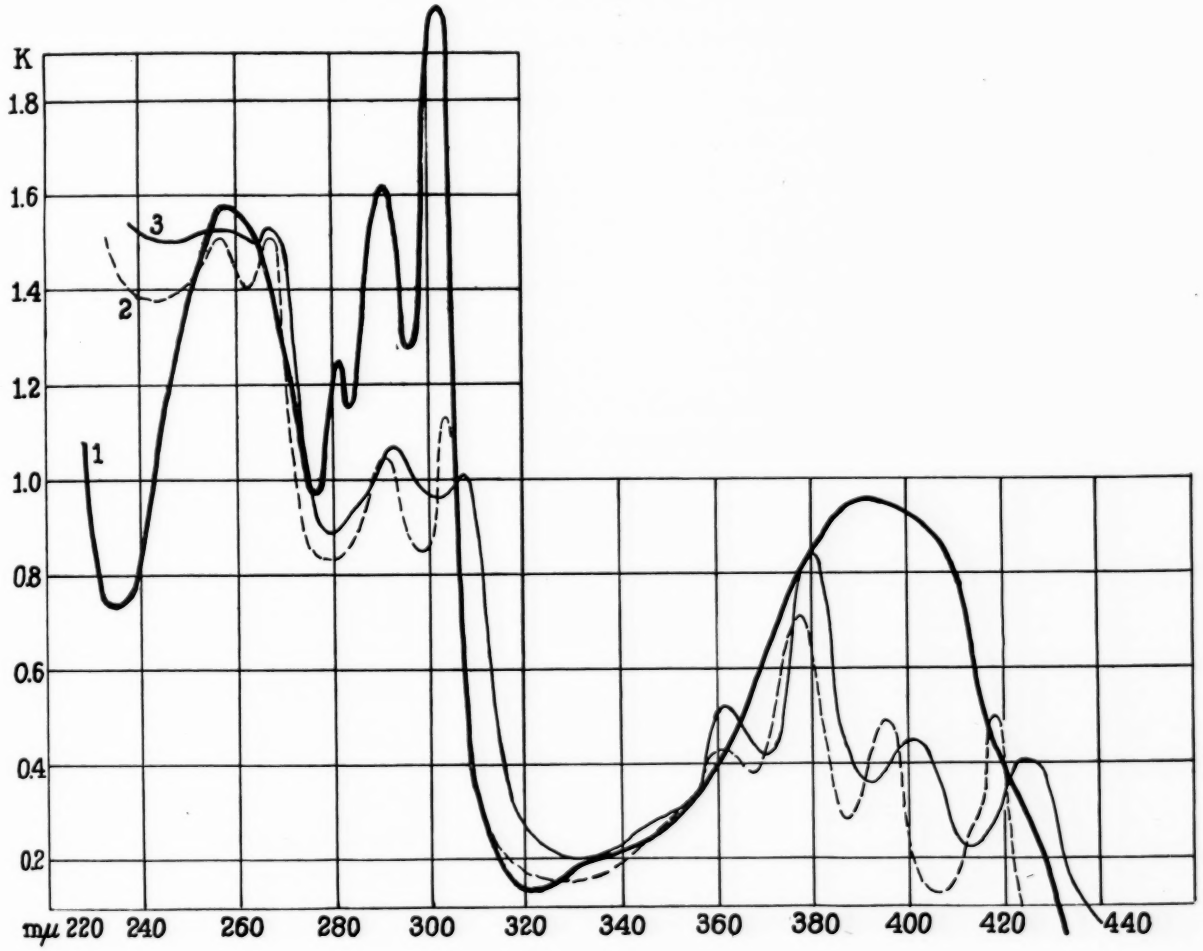


Fig. 3.—Absorption spectra.
1. 5-Hydroxybenzpyrene in ethanol (Fieser).
2. BPX in hexane.
3. BPX in ethanol.

approximate to right angles and the *a* and *c* axes are practically indistinguishable in length. The near identity in the two cell dimensions extends to the intensities of corresponding x-ray reflections, and suggests that the arrangement of the molecules in the *b* plane has symmetry which is destroyed in the crystal structure as a whole by staggering of the planes.

The unit cell dimensions may be given as shown in Table II. The cell dimensions are probably correct to 1 to 2 per cent. Since in the case of methylated BPX only small quantities of material were available, the crystal densities were determined by flotation in drops of solutions of known densities under the microscope, as in the case of BPX itself (3). Three solutions, of densities 1.332, 1.361, and 1.396, were prepared and the behavior of the crystals in these liquids is indicated in Table II. $M_{calc.}$ in these figures is derived from the best values of the unit cell dimensions and the outside limits for the density. Possible experimental errors in the x-ray measurements certainly widen the permitted limits for the measured molecular weight. But the good agreement between $M_{calc.}$ and $M_{theor.}$ proves clearly that methylated BPX is a monomethoxybenzpyrene, while it confirms the dimethoxy character of the quinone derivative.

The fact that the crystal structures of methylated BPX and 5,8-dimethoxybenzpyrene are so similar is presumably correlated with the presence of the methoxy group in methylated BPX at the same position as one of those in the dimethoxy compound. That this should be the 8- rather than the 5- position is in good agreement with the observations made on BPX itself (3).

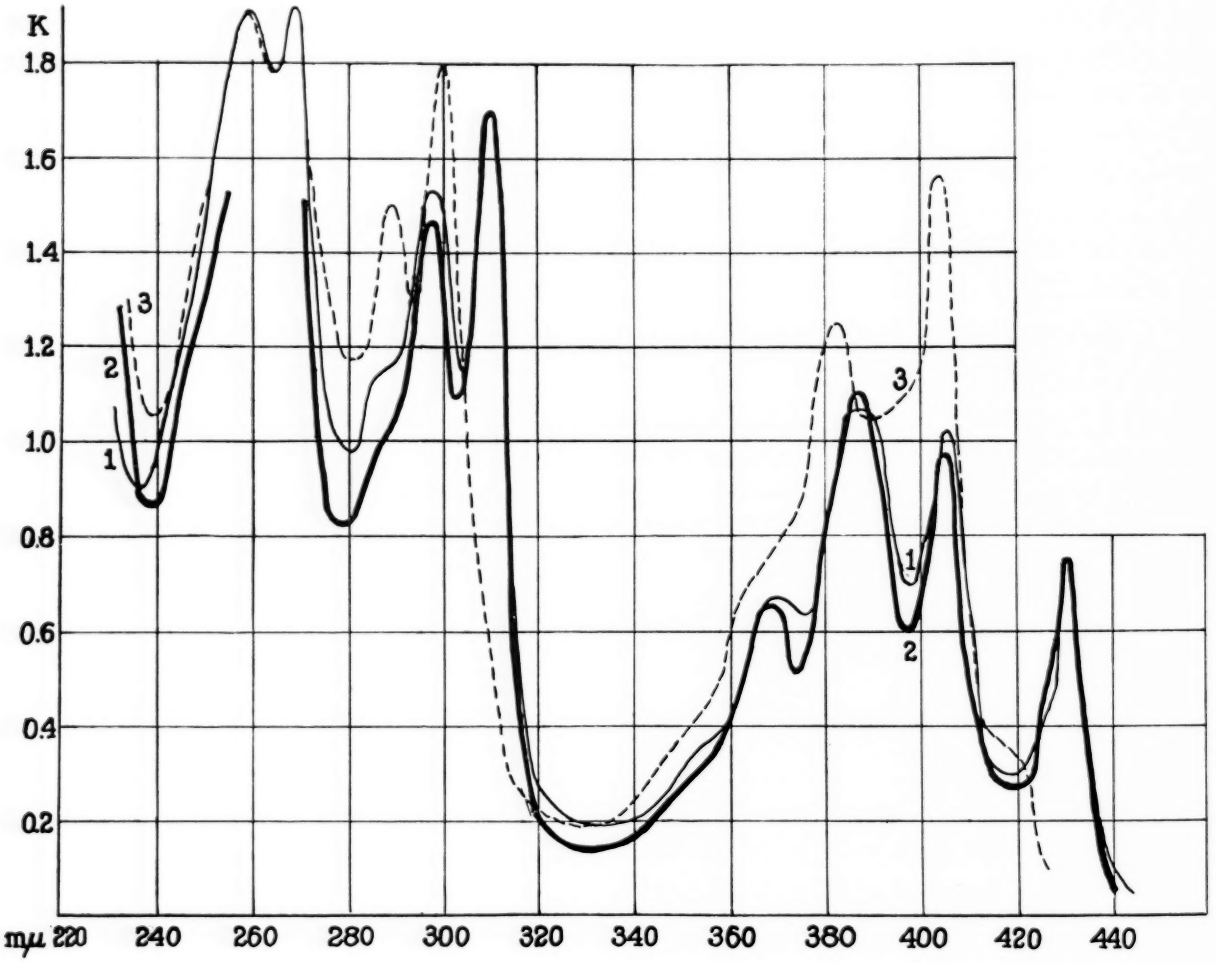


FIG. 4.—Absorption spectra.
1. Methoxy derivative of red substance from the feces.
2. 5,8-Dimethoxy-3,4-benzpyrene.
3. 5,10-Dimethoxy-3,4-benzpyrene.

DISCUSSION

The evidence of the foregoing results leads to the following conclusions: (a) the nonidentity of methylated BPX with the methoxy compounds derived from benzpyrene-quinones; (b) the identity of the methoxy compound derived from the red product from the

feces with that from the synthetic 5,8-quinone of benzpyrene; and (c) the allocation of the hydroxy group in BPX to the 8- position.

TABLE I

	Morphology	Optics
1. 5,10-Dimethoxybenzpyrene	Light yellow laths, probably monoclinic or orthorhombic	Extinction straight
2. 5,8-Dimethoxybenzpyrene	Yellow needles or prisms, triclinic, showing {100}, {001}; elongated along [010]; frequently twinned	Extinction oblique, "slow" direction, 46-50° to needle axis
3. Methylated BPX	Yellow needles, triclinic, showing {100}, {001}; elongated along [010]	Extinction oblique, "slow" direction c, 42° to needle axis

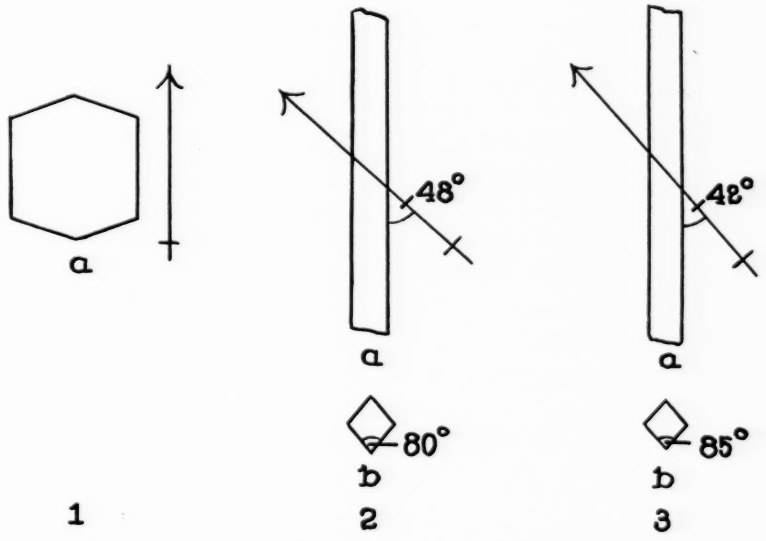


FIG. 5.—Diagram to illustrate the optic and morphological character of crystals of:
1. 5,10-Dimethoxy-3,4-benzpyrene.
2. 5,8-Dimethoxy-3,4-benzpyrene.
3. Methylated BPX.

The "slow" extinction direction in each case is shown by the arrows: (a) normal view in microscope, (b) needle cross-section.

TABLE II: UNIT CELL DIMENSIONS (X-RAY MEASUREMENTS)

	Methylated BPX	5,8-Dimethoxy-benzpyrene
a	18.72 Å	19.0 Å
c	18.7 Å	19.0 Å
$d_{(010)}$	8.01 Å	8.52 Å
β	95°	100°
	Space group P_1 or $P\bar{1}$ $n = 8$	
ρ	1.322–1.361	1.361–1.396
$M_{calc.}$	276–286	310–318
$M_{theor.}$	282 for $C_{20}H_{11}OCH_3$	312 for $C_{20}H_{10}(OCH_3)_2$

(a) Methylated BPX clearly differs from any of the methoxy compounds derived from the synthetic quinones, since its fluorescence and absorption spectra and crystal structure are all different, while both the analytical data for methoxy groups and the x-ray measurements agree in establishing that the former is a mono- and the latter are dimethoxy derivatives of benzpyrene. From this it is evident that BPX itself is a monohydroxybenzpyrene, in confirmation of the findings of Chalmers and Crowfoot (3).

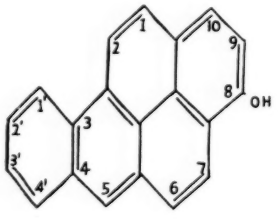
(b) The identity of the red product from the feces with 3,4-benzpyrene-5,8-quinone, already suggested by its general properties (1), is established by the identity of the fluorescence and absorption spectra and the crystal structures of their methylated derivatives, and by the demonstration that the methylated derivative of the 5,10-quinone possesses entirely different spectra and crystalline form.

(c) Since on oxidation BPX yielded a red substance which, on reductive methylation, produced a methoxy compound indistinguishable from the 5,8-methoxybenzpyrene, it may be concluded that BPX itself is either 5-hydroxy- or 8-hydroxybenzpyrene, and that

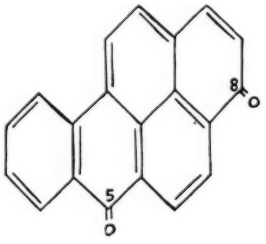
an intermediate 5,8-dihydroxybenzpyrene stage is probably involved in the conversion. (The difficulty of isolating this intermediate product is understandable, in view of the observed instability in air of the reduction products of benzpyrene-quinones.) However, 5-hydroxybenzpyrene is excluded by the pronounced difference between the absorption spectrum of synthetic 5-hydroxybenzpyrene (4) and that of BPX. Further, 5-hydroxybenzpyrene would be expected to yield on oxidation a mixture of 5,8- and 5,10-quinones but, as shown above, oxidation of BPX yields entirely 5,8-quinone. The hydroxy group in BPX is therefore allocated to the 8- position. Thus the prediction of Cason and Fieser (2), that the hydroxy group in the metabolic derivative of benzpyrene is not likely to be in the 5- position, is substantiated.

SUMMARY

A study has been made of the crystallography and the fluorescence and absorption spectra of certain derivatives of the two products of 3,4-benzpyrene isolated from mouse and rat feces, and these data have been compared with similar data from comparable derivatives of synthetic compounds.



8-hydroxy-3,4-benzpyrene



3,4-benzpyrene-5,8-quinone

By these means it was possible to identify the red product with 3,4-benzpyrene-5,8-quinone, and to arrive at the conclusion that the fluorescent product BPX is 8-hydroxy-3,4-benzpyrene.

We wish to thank Mr. W. Weinstein and Mr. H. W. Wheal for valuable technical assistance.—AUTHORS.

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ADDENDUM

After this paper had been submitted for publication, direct comparisons were made between the methylated product of the metabolic hydroxy derivative of benzpyrene and 5-methoxy-3,4-benzpyrene, synthesized by the method of Fieser and Hershberg (4). The two were found to be different as regards their crystal structure and their absorption and fluorescence spectra, thus confirming the conclusions already reached.

The Glycerophosphatases of the Rat Liver Cancer Produced by Feeding *p*-Dimethylaminoazobenzene

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(Received for publication September 21, 1942)

The metabolism of the liver tumors produced in rats by the feeding of *p*-dimethylaminoazobenzene has recently been studied extensively. In particular it has been shown that the diphosphopyridine nucleotide content of the livers of rats fed this compound is greatly decreased, and that the metabolites of the dye are toxic to fermenting systems in which diphosphopyridine nucleotide is the limiting factor (5–7). In the course of another investigation (13, 14) the author found that the alkaline glycerophosphatase content of some types of human cancer is much greater than that of analogous normal tissues, and that the phosphatases of supposedly normal human livers are somewhat variable. It therefore seemed desirable to determine whether the glycerophosphatase activity of the livers of rats fed *p*-dimethylaminoazobenzene showed any changes that could be related to the other metabolic abnormalities known to be present. The relation of liver phosphatase to the storage of phosphorus was also studied, radioactive phosphorus being used as an indicator. After this work was nearly completed, Greenstein (2) reported a similar study of the phenylphosphatase of liver tumors. A comparison of the action of extracts of liver tumors on two different phosphate esters is thus made possible.

MATERIAL AND METHODS

The rats were young adult albino animals of both sexes, kindly supplied by Dr. K. Sugiura, of this hospital. They were fed a diet consisting of 20 cc. of a 3 per cent solution of *p*-dimethylaminoazobenzene in olive oil mixed with 1,000 gm. of brown rice, and supplemented with carrots. Controls were fed either on the same diet without the dye or on a stock diet of Purina chow and carrots or lettuce. No differences were observed between the two control groups.

After 36 to 272 days on the experimental diet the animals were killed by a blow and the livers were removed and inspected. If the liver was homogeneous, the entire organ was minced with scissors for phosphatase and radioactive phosphorus determinations. If the liver contained both cancerous areas and relatively normal tissue, the different portions were dissected and handled separately. Representative specimens were taken for histological examination before the remainder of the tissue was minced.

Macro-phosphatase determinations were made by the method described in detail elsewhere (14). Micro-phosphatase determinations were made at pH 4 to 5 and at pH 8.8 to 9.2 by a method based on those of Gomori (1) and of Kabat and Furth (4). Radioactive phosphorus was administered by the subcutaneous injection of a solution containing 5 μc. P^{32} and 0.15 to 0.30 mgm. $Na_2HP^{31}O_4$. The radioactivity of the ashed specimens was determined by methods previously employed in this hospital (9).[1] The total phosphorus of the ash was determined by a modification of the methods of Pregl and Roth (10) and of Scott (11).

TABLE I

Tissue	Number of determinations	Acid P-ase, units per gm.		Alkaline P-ase, units per gm.	
		Average	Range	Average	Range
Liver cancer	11	2.5	0.64–9.8	1.6	0.40–5.0
Precancerous liver	10	2.0	1.1 –3.0	0.39	0.11–0.85
Normal liver	8	1.7	0.45–3.0	0.16	0.10–0.28

RESULTS

The results of the macro-phosphatase determinations are summarized in Table I. In all the liver specimens of the first group there was definite evidence of cancer, but some consisted almost entirely of cancer while others contained cancer nodules mixed with varying amounts of abnormal but noncancerous liver. The specimens of the second group showed precancerous changes with large hyperchromatic nuclei and bile

[1] We are indebted to Dr. John H. Lawrence, of the University of California, for supplying us with the radioactive phosphorus used, and to Mr. L. D. Marinelli, of the Memorial Hospital, for making the radioactivity measurements.

duct adenomas. The specimens in the third group were normal appearing livers from normal animals. Only a few of them were examined histologically.

The average value for acid phosphatase in the liver cancers is a little higher than for the normal livers, but as there is a wide range within each group and considerable overlapping between groups the difference cannot be considered significant. In the alkaline phosphatase, however, significant differences do appear. The average value for the alkaline phosphatase of the liver cancers is 1.6 units per gm., or 10 times the average for the normal livers. The range of alkaline phosphatase readings on the liver cancers is wide, but the lowest is higher than the highest reading in the normal group. The alkaline phosphatase values of the precancerous group occupy an intermediate position, overlapping both the cancers and the normal livers.

These figures suggest that the increased production or retention of alkaline phosphatase began in the damaged livers before definite malignant changes appeared. Unfortunately, there is some uncertainty on this important point, since small cancer nodules may have been included in the large specimens taken for phosphatase determination, but not in the small ones selected for histological examination. In some specimens the appearance of precancerous changes detectable under the microscope is known to have preceded the rise in alkaline phosphatase. One of these is illustrated by Fig. 1, C.

The differences between the phosphatase activities of normal and pathological livers are shown graphically in Fig. 1. In this figure the phosphatase activities are plotted against the pH at which the determinations were made. Curve A shows the behavior of an extract of the liver of a normal rat. There is considerable acid phosphatase with a broad maximum of activity between pH 4.0 and 5.0 and a great diminution below pH 3.0. The alkaline phosphatase activity is low. Curves B and C show two portions of the liver of a rat that had been fed a *p*-dimethylaminoazobenzene diet for 272 days. Specimen B was almost entirely cancer; specimen C showed early precancerous changes. The curves for specimens A and C are very similar. The alkaline phosphatase activity of the liver cancer shown in curve B is 7 to 8 times that of the other specimens, and has a sharp maximum at about pH 9.7.

When it became evident that the liver cancers contained large amounts of alkaline phosphatase, an attempt was made to discover which cellular elements produced this enzyme. Micro-phosphatase determinations were accordingly made on some of the specimens. Dr. F. W. Stewart, of this hospital, kindly reviewed the slides and reported that acid phosphatase was present in the nuclei and in the cytoplasm of the tumor cells and in the nuclei of the connective tissue cells. Alkaline phosphatase was present in the intercellular spaces of many specimens, though this probably did not represent tissue phosphatase but rather the phosphatase activity of small amounts of bile included in the preparation. Alkaline phosphatase was not demonstrated in any of the normal liver cells, as was to be expected from the very low alkaline phosphatase readings on extracts of normal liver. In the liver cancers, alkaline phosphatase was present mainly in the endothelial cells of the sinuses, but could also be seen in the nuclei and possibly also in the cytoplasm of the tumor cells.

As the function of the alkaline phosphatase in the metabolism of liver cancer is unknown, it seemed desirable to determine whether this enzyme promoted the storage of phosphorus. This was done by making subcutaneous injections of a solution of radioactive phosphorus into normal and *p*-dimethylaminoazobenzene-fed rats and measuring the radioactivity of their livers at various times thereafter. It is known from the work of numerous investigators that when radioactive phosphorus is administered to animals the isotope is taken up very rapidly by the liver, but that a considerable portion is lost rather promptly. The initial rapid uptake is probably due to the incorporation of the radioactive phosphorus in various phosphorus-containing compounds, especially phospholipids, which are elaborated in the liver. These are not stored permanently at the site of formation, but are transported to other parts of the body. The smaller amounts of radioactive phosphorus remaining in the liver after the initial rise and drop probably represent true intracellular phosphorus. In order to study both types of compounds, some of the animals were killed 48 hours after injection, and the remainder 96 hours after injection. The average differential absorption ratios at 48 hours were 1.21 to 1.24, and at 96 hours were 0.81 to 0.96. There were no significant differences between the normal livers, the livers that had undergone precancerous changes, and the liver cancers. Only three animals were included in each group, as it was evident early in the work that large differences between the groups would not be observed.

For comparison with the livers, radioactive phosphorus determinations were also made on the kidneys, thigh muscles, and femurs of the same animals. The total phosphorus (P^{31}) content of the ash of the specimens was also measured. Results for the total phosphorus of liver, kidney, and muscle, and for the radioactive phosphorus of kidney and muscle were rather variable, so that it was not apparent whether there were significant differences between the normal and the dye-fed animals. There was some evidence that

the total phosphorus content of the bones was less, and the uptake of radioactive phosphorus by the bones was slower, in the rats with damaged livers than in the normal animals. This may have been due to the poor nutritional state of the dye-fed animals, with diversion of the available phosphorus supplies from the bones to other tissues where it is needed more urgently. Similar findings on the bones of tumor-bearing animals have been made by other investigators (8).

Alkaline glycerophosphatase thus is present in large quantities in liver cancers produced by feeding *p*-dimethylaminoazobenzene, but is not concerned in the storage of phosphorus in the tissue. Possibly this enzyme is substituted for some mechanism in the carbohydrate cycle that is inactivated by such split products. Dimethyl-*p*-phenylenediamine is a probable metabolic product of *p*-dimethylaminoazobenzene whose inhibiting effect on fermenting systems has been demonstrated clearly (6). This substance interferes with the analytical methods for phosphatase only when it is kept long enough in alkaline solution to develop an intense purple color. Its effect on phosphatase preparations can therefore be determined readily, except when the alkaline phosphatase activity is so low as to require 2 hours or more of incubation. Accordingly, we studied the effect of $M/1{,}000$ dimethyl-*p*-phenylenediamine on the acid and alkaline β-glycerophosphatases of one normal rat liver and of two rat

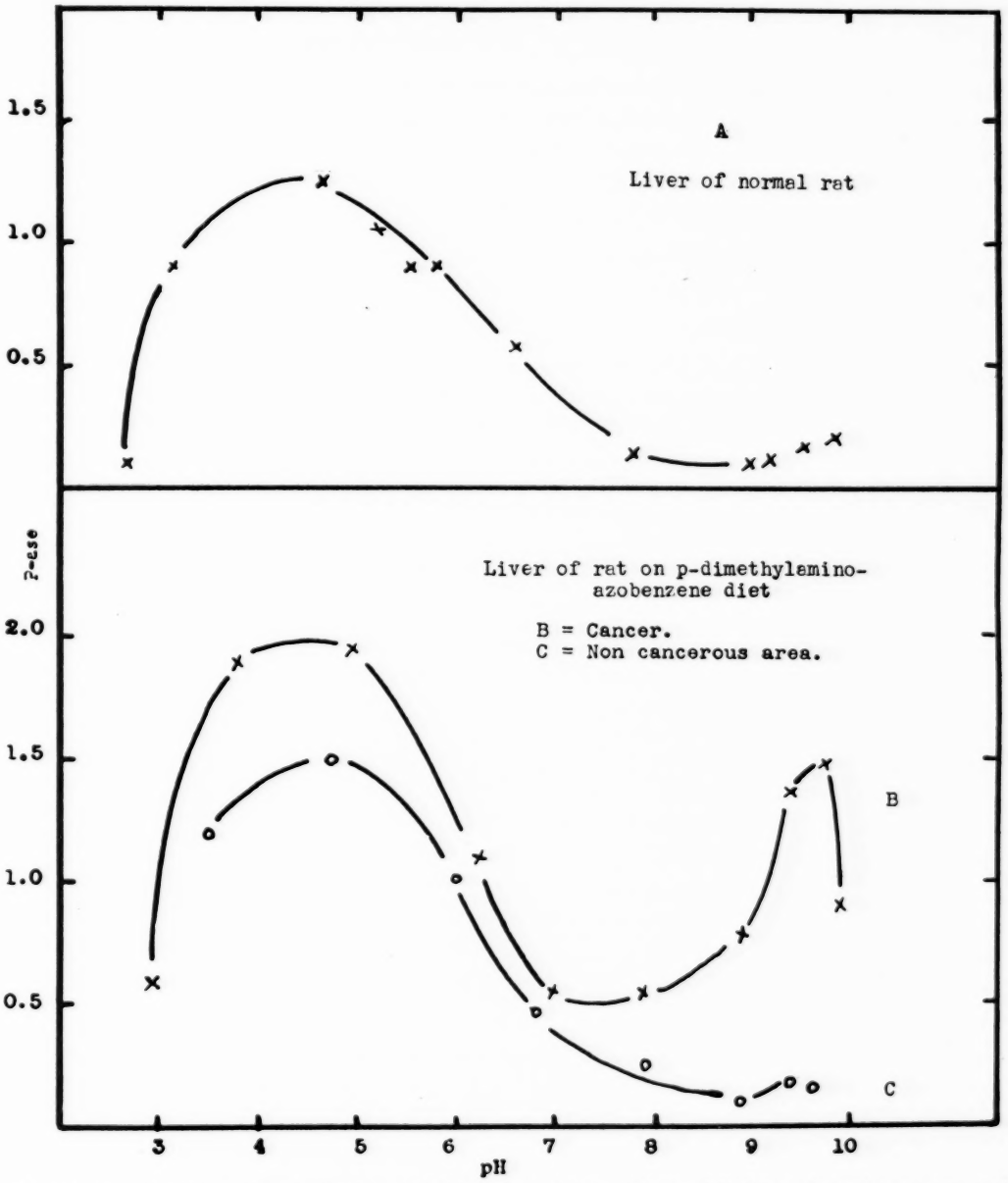

FIG. 1.—Phosphatase of extracts of normal and pathological rat livers. Tissue phosphatase in units per gram plotted against the pH at which the determinations were made.

liver cancers. For comparison we also studied its effect on the acid phosphatase of normal and hypertrophied prostate glands, and on the alkaline phosphatase of a low grade chondrosarcoma. The apparent effect of dimethyl-*p*-phenylenediamine ranged from 7 per cent inhibition to 15 per cent activation. Such differences are within the experimental error of the method for tissue phosphatase, which, under unfavorable conditions, may equal ±25 per cent. We may therefore conclude that dimethyl-*p*-phenylenediamine in a concentration of *M*/1,000 has no effect on the phosphatases examined.

DISCUSSION

The evidence presented above shows that the alkaline β-glycerophosphatase activity of rat liver cancers produced by the feeding of *p*-dimethylaminoazobenzene averages 10 times that of normal rat livers. The appearance of excess enzyme probably precedes that of definite malignant changes, but does not take place until degeneration is advanced. The enzyme is present in the nuclei of the tumor cells. It is not inactivated by a probable split product of *p*-dimethylaminoazobenzene that has a strong inhibitory effect on one phase of the carbohydrate cycle.

The demonstration of the presence of large amounts of alkaline glycerophosphatase in primary rat liver tumors induced by feeding *p*-dimethylaminoazobenzene is in harmony with the observations of Greenstein (2) on the phenylphosphatase of transplanted rat liver tumors of a strain originally induced by a diet that included *p*-dimethylaminoazobenzene. The enzyme is thus not specific for any one phosphate ester. The inability of our tumors to store more radioactive phosphorus than normal livers is not in harmony with the observation of White, Dalton, and Edwards (12) that rat hepatoma 31 frequently calcifies. It is possible that this characteristic of the tumor may become altered somewhat on transplantation.

This occurrence of much larger alkaline β-glycerophosphatase activities in cancer than in analogous normal tissue is not an isolated phenomenon. We have shown elsewhere (14) that the alkaline phosphatase activities of embryonal carcinoma of the testis and of myogenic sarcoma may be much higher than those of normal testis and normal muscle respectively. The high alkaline phosphatase activity of osteogenic sarcoma is well known, and is associated with the rapid storage of phosphorus. We have shown, however, that the alkaline phosphatase of osteogenic sarcoma is not nearly so effective per unit of activity in promoting the storage of phosphorus as is that of normal bone, and that the more malignant the tumor the less effective is the enzyme (13). Recently Gutman, Warrick, and Gutman (3) have reported that calcifying cartilage contains a mechanism for phosphorylative glycogenolysis, and that the ester so produced probably serves as the substrate for bone phosphatase. It is possible that in osteogenic sarcoma this mechanism is inactivated, and that excessive amounts of alkaline phosphatase are formed in an attempt at compensation. While comparisons between different tissues are not necessarily valid, it is permissible to speculate whether a similar situation may exist in liver cancer. Some mechanism which normally furnishes substrate for the small amounts of alkaline phosphatase in normal liver may become inactivated by the metabolic products of *p*-dimethylaminoazobenzene. Since the activity of the phosphatase itself is unaffected by these toxic products, the production of phosphatase may be increased as a compensatory mechanism.

SUMMARY

The alkaline β-glycerophosphatase activity of the liver cancer produced in rats by the feeding of *p*-dimethylaminoazobenzene averages 10 times that of normal rat liver.

Alkaline β-glycerophosphatase can be demonstrated in the endothelial cells of the sinuses of the liver cancers and in the nuclei of the tumor cells.

The storage of radioactive phosphorus in liver cancer is the same as that in normal liver 48 and 96 hours after injection of the isotope.

Neither the acid nor the alkaline β-glycerophosphatases of normal rat liver or rat liver cancer are inhibited by dimethyl-*p*-phenylenediamine, one of the probable metabolic products of *p*-dimethylaminoazobenzene.

The possible significance of these findings is discussed.

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Metabolism of Yale Carcinomal *

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Yale carcinoma 1 arose originally as an adenocarcinoma of the mammary gland in a male mouse of Strong's A strain following treatment with estrogen (7), and has been maintained by transplantation for the past several years by a number of investigators throughout the country. Since it is being increasingly used in experimental cancer work (1, 8, 11, 13, 14, 17), it was thought that a knowledge of its metabolic properties would be desirable.

APPARATUS AND TECHNIC

Simple Barcroft-Warburg manometers (4) with conical vessels were employed for determining the respiration and anaerobic glycolysis, and the same manometers, but with rectangular flasks, for aerobic glycolysis. The respiratory quotient was obtained by the use of Summerson manometers (16) with Dixon-Keilin vessels (4).

Slices 0.2 to 0.3 mm. thick were cut freehand with a razor from freshly excised tumor selected for freedom from necrosis. This and other preparatory procedures were usually carried out in the media used for the experiment. However, if the effect of a particular substrate was being studied, the tissue was kept in Krebs-Henseleit phosphate solution or 0.9 per cent NaCl until placed in manometer vessels containing the experimental solution. Portions of a number of the tumors used in this work were fixed and sectioned for histological study. Microscopic examination showed that they were adenocarcinomas, entirely similar in structure to the original tumor (7).

EXPERIMENTAL

Respiration

The results of the experiments on the rate of oxygen consumption are given in Table I. As indicated, the Q_{O_2} values for this tumor in *air* and with *M*/50 phosphate buffer or the Krebs-Henseleit phosphate solution as medium (9) were about −2. In an atmosphere of *pure oxygen* the respiration more than trebled, to yield Q_{O_2} values of about −7. The addition of substrates such as glucose, fructose, lactate, or pyruvate (0.2 per cent in each case) in air or 100 per cent oxygen was without effect, giving no significant increase in respiration beyond that observed in the corresponding "atmosphere" without substrate.

Table I: Respiration of Yale Carcinoma 1

Medium	Atmosphere	Substrate	Number of vessels	Q_{O_2} values		
				Minimum	Maximum	Average
M/50 phosphate	Air	0	15	−1.3	−2.6	−2.0
Krebs-Henseleit phosphate	Air	0	12	−1.4	−2.9	−2.2
Krebs-Henseleit phosphate	100% O_2	0	12	−5.6	−8.5	−7.3
M/50 phosphate	Air	0.2% glucose	4	−1.3	−1.9	−1.6
M/50 phosphate	Air	0.2% fructose	4	−1.7	−2.6	−2.1
Krebs-Henseleit phosphate	100% O_2	0.2% glucose	2	−6.2	−6.2	−6.2
Krebs-Henseleit phosphate	100% O_2	0.2% lactate	2	−8.0	−9.2	−8.6
Krebs-Henseleit phosphate	100% O_2	0.2% pyruvate	2	−6.7	−8.9	−7.8

Respiratory Quotient

The respiratory quotient [1] was determined by the use of Summerson manometers and Dixon-Keilin vessels. The R. Q. values, in phosphate medium and in an atmosphere of 100 per cent oxygen, gave an average of 0.80.

It is noteworthy that the addition of substrate in the form of 0.2 per cent glucose had no effect on the respiratory quotient; *i.e.*, gave values identical with

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[1] We are indebted to Miss Virginia C. Dickerson, of the Department of Physiological Chemistry, for her aid in these experiments.

those for the phosphate medium without substrate. The results of these experiments are given in Table II.

Glycolysis

Aerobic glycolysis.—Warburg's indirect method (4, 18), employing two vessels, was used in determining the aerobic glycolysis of the tumor. The vessels were of the rectangular type, coupled with simple Barcroft-Warburg manometers. The atmosphere employed was 95 per cent O_2 and 5 per cent CO_2, the total volume of the vessels approximately 11.5 cc. The fluid volumes used were 1 cc. and 5 cc., yielding vessel constants for oxygen and carbon dioxide of $K_{O_2}=0.79$ and 0.74, $K_{CO_2}=0.85$ and 0.99, respectively.

In computing the values for aerobic glycolysis the respiratory quotient of 0.8, found for this tumor in our experiments, was used instead of assuming a respiratory quotient of 1.0. The results are given in Table III.

Anaerobic glycolysis.—Barcroft-Warburg manometers with conical vessels were employed to determine the anaerobic glycolysis in an atmosphere of 95 per cent N_2 and 5 per cent CO_2, with 0.2 per cent glucose as substrate. The range of values obtained yielded an average for $Q_G^{N_2}$ of about 16 (Table IV).

Table II: Respiratory Quotient of Yale Carcinoma 1

Medium	Atmosphere	Substrate	Number of vessels	R. Q. values		
				Minimum	Maximum	Average
Krebs-Henseleit phosphate	100% O_2	0	2 pairs	0.73	0.84	0.80
Krebs-Henseleit phosphate	100% O_2	0.2% glucose	2 pairs	0.73	0.88	0.81

Table III: Respiration, Aerobic, and Anaerobic Glycolysis of Yale Carcinoma 1

Medium	Atmosphere	Substrate	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	Meyerhof quotient $\frac{Q_G^{N_2}-Q_G^{O_2}}{Q_{O_2}}$
Krebs-Henseleit bicarbonate	95% O_2 5% CO_2	0.2% glucose	−5.4	7.5	15.2	1.4
Krebs-Henseleit bicarbonate	95% O_2 5% CO_2	0.2% glucose	−6.3	7.2	16.0	1.4
Krebs-Henseleit bicarbonate	95% O_2 5% CO_2	0.2% glucose	−5.8	5.3	18.1	2.2
Krebs-Henseleit bicarbonate	95% O_2 5% CO_2	0.2% glucose	−5.2	5.6	16.3	2.1
Krebs-Henseleit bicarbonate	95% O_2 5% CO_2	0.2% glucose	−5.8	6.7	17.1	1.8
Krebs-Henseleit bicarbonate	95% O_2 5% CO_2	0.2% glucose	−8.0	7.0	18.8	1.5
		Average	−6.1	6.6	16.9	1.7

Table IV: Anaerobic Glycolysis of Yale Carcinoma 1

Medium	Atmosphere	Substrate	Number of vessels	$Q_G^{N_2}$ values		
				Minimum	Maximum	Average
Krebs-Henseleit bicarbonate	95% N_2 5% CO_2	0.2% glucose	18	12.0	19.6	15.7

Discussion

Comparison of Yale Carcinoma 1 with Other Tumors

In order to compare the metabolic properties of Yale carcinoma 1 with those of other transplantable tumors,

Table V has been compiled. The data for the other tumors were selected from reports published by other workers. The values obtained in the present investigation are in general agreement with those reported for other tumors by Burk, Crabtree, Murphy and Hawkins, Warburg, Dickens, and others. Thus Yale carcinoma 1 exhibits an appreciable aerobic and a fairly high anaerobic glycolysis and has a respiratory quotient which is definitely lower than that of normal tissues in the presence of carbohydrate. It should be emphasized that within the experimental periods employed neither the rate of oxygen uptake (Q_{O_2}) nor the respiratory quotient is significantly affected by the absence or presence of substrate in the medium. It is inferred that the tumor slices subsist on a substrate reserve stored in the tissue.

With regard to oxygen consumption, Yale carcinoma 1 resembles more closely the Flexner-Jobling rat carcinoma or the Jensen rat sarcoma than most of the mouse tumors that have been studied. However, there is one instance of a melanotic sarcoma that is strikingly similar to the Yale carcinoma 1 in its metabolic properties (Table V).

Table V: Comparison of Metabolic Properties of Yale Carcinoma 1 with Those of Other Tumors

Tumor	Species	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	Meyerhof quotient $\frac{Q_G^{N_2} - Q_G^{O_2}}{Q_{O_2}}$	Reference
Yale carcinoma 1	*Mouse*	— 7	7	*16*	*1.7*	*Present experiments* *
Glycogen carcinoma 113	Mouse	—11	4	14	0.9	Crabtree (3)
Tar carcinoma 2146	Mouse	—20	15	25	0.5	Crabtree (3)
Sarcoma 37	Mouse	—14	12	29	1.2	Crabtree (3)
Crocker sarcoma	Mouse	—16	17	28	0.9	Crabtree (3)
Melanotic sarcoma	Mouse	— 8	6	16	1.3	Crabtree (3)
Flexner-Jobling carcinoma	Rat	— 8	20	29	1.1	Murphy and Hawkins (12)
Flexner-Jobling carcinoma †	Rat	— 7	25	31	0.9	Burk (2)
Jensen sarcoma	Rat	— 9	18	32	1.6	Crabtree (3)

* The figures for Yale carcinoma 1 presented here are average values derived from the experiments reported in this communication.
† Referred to by Burk as Flexner-Jobling sarcoma.

Gas Affinities and Pasteur Effect

When the oxygen concentration was increased from 20 to 100 per cent, the respiration of thin slices of Yale carcinoma 1 was more than trebled, which can hardly be ascribed to the thickness of the slices since these were well within the range used by other authors. Laser (10) and others have found no change in the oxygen uptake of normal and tumor tissues upon decreasing the oxygen tension from 100 to 20 per cent or even lower. The present observations suggest that the respiratory ferment in the cells of Yale carcinoma 1 has a relatively low affinity for oxygen and that it is no longer saturated with the gas or, rather, that it no longer exists predominantly in the ferriform at a partial oxygen pressure of about 150 mm. of mercury. The tissue exhibits a definite Pasteur effect in that the admission of oxygen (95 per cent) will decrease the rate of lactic acid formation (glycolysis) to less than one-half the anaerobic level. The Meyerhof quotient as defined by Warburg (anaerobic glycolysis − aerobic glycolysis ÷ respiration) is 1.4 to 2.2, and therefore on the lower side of the normal range (1 to 6).

Respiration of Yale Carcinoma 1 and Its Cytochrome Content

It has been asserted by von Euler (6) and others that certain experimental tumors have an abnormally low cytochrome content and that this fact is in causal connection with their atypical metabolism, especially their aerobic glycolysis. Some time ago one of us (K. G. S.) observed directly fairly strong cytochrome absorption bands in slices of Yale carcinoma 1 (15), a fact which seemed to militate against von Euler's hypothesis. Recently DuBois and Potter (5) have made quantitative estimations of the cytochrome *c* concentration of this tissue and have found it to possess 16γ per gm. on the average. This value is relatively high when compared with other tumors, but relatively low when compared with most normal tissues, and of about the same range as that found in lung. As is shown in this report, Yale carcinoma 1 exhibits a metabolism rather typical for transplantable tumors in spite of its fairly appreciable cytochrome *c* content. The present findings therefore fail to support von Euler's hypothesis, but since the aerobic glycolysis of this tumor is on the low side, as compared with certain other tumors, these findings do not represent decisive evidence against this theory.

Summary

Some of the metabolic properties of Yale carcinoma 1 have been studied. This tumor is a transplantable adenocarcinoma of the mammary gland that arose in a male mouse following the administration of estrogen.

It was found to have on the average a Q_{O_2} of -2 in air, a Q_{O_2} of -7 in 100 per cent oxygen, an aerobic glycolysis of 7, and an anaerobic glycolysis of 16. The respiratory quotient was 0.80 and the Meyerhof quotient was 1.4 to 2.2. These data suggest that the respiratory ferment in this tumor has a relatively low affinity for oxygen.

The tumor exhibits a definite Pasteur effect.

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The Effect of 2,7-Dihydroxynaphthalene on Tumor Incidence and Growth of the Mammary Gland in Mice of the Line A Albino and C3H Strains*

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The effect of subcutaneous injections of 2,7-dihydroxynaphthalene on the growth of the mammary gland and the formation of its tumors in mice of the C3H and line A albino strains has been investigated. These experiments were undertaken to discover whether the "sparing effect" exerted on estrogens by this chemical would enhance or delay the production of tumors when 2,7-dihydroxynaphthalene was given in addition to estrogen.

The activity of 2,7-dihydroxynaphthalene was first described by Pincus (3), who reported that in combination with progesterone stimulation of uterine gland proliferation and growth of ova occurred. He suggested that the action was accomplished through inhibition of progesterone destruction. Naphthalene was also shown to have a definite stimulating effect on the endometrium and was therefore tested for progesterone-like activity. From his experiments he concluded that 2,7-dihydroxynaphthalene is probably not a progestin but rather "a presumable degradation product of estrogen of such chemical nature that it still retains a certain amount of estrogenic activity." Pincus and Werthessen (4) analyzed the estrogenic activity and found that 2,7-dihydroxynaphthalene had 0.14 per cent of the activity of estrone. Smith, Smith, and Schiller (5) suggested that 2,7-dihydroxynaphthalene "enhanced the estrogenic activity of estrone upon the vaginas of spayed rats probably through a protective action . . . against estrogen destruction."

On the assumption that 2,7-dihydroxynaphthalene exerts a "sparing effect" on estrogen by preventing degradation of the hormone, it might be expected that mice receiving this chemical in addition to a given amount of estrogen would react as though they had received a larger amount of estrogen and fail to show the customary reactions to estrogen breakdown products. Thus, if mammary gland tumors develop in direct response to estrogen stimulation, mice injected with naphthalene in addition to the hormone should develop tumors at an earlier age and in a higher percentage than those given hormone alone. Conversely, if tumors are produced in response to the presence of degradation products of estrogen metabolism, mice receiving naphthalene plus the hormone should develop tumors at a later age and in fewer instances, since naphthalene apparently prevents this degradation to some extent.

MATERIALS AND METHODS

Normal and ovariectomized female mice of the C3H strain were used for all experiments on the effect of 2,7-dihydroxynaphthalene on estrus, growth of the mammary glands, and spontaneous tumor formation. Normal male mice of the C3H strain and normal and castrated males from the line A albino strain were used in the experiments on the effect of naphthalene on the growth of male mammary glands and on tumor formation following injections with estrogen. All mice were from stocks bred in this laboratory. The original C3H stock was received from Dr. H. B. Andervont, and the original line A albino stock from the Roscoe B. Jackson Laboratory at Bar Harbor. All mice were fed the stock diet of Purina fox chow and water ad libitum.

The estrogen used was estradiol benzoate, progynon B, the benzoic acid ester of dihydrofollicular hormone in oil, prepared by the Schering Corporation. This was diluted with olive oil so that each 0.05 cc. contained 50 rat units. Injections were made subcutaneously.

The 2,7-dihydroxynaphthalene was obtained from the Eastman Kodak Company. It was prepared by dissolving 800 mgm. in ether to which was added 20 cc. of olive oil. The ether was then evaporated. The resulting solution was supersaturated and crystals of

* This investigation was aided by The Reginald W. Bird endowment for research in cancer.

naphthalene formed at the bottom of the container. It was therefore impossible to say exactly how much of the chemical was contained in each cubic centimeter, but it obviously could not have exceeded 40 mgm. The naphthalene was given subcutaneously, since it is impossible to give long-continued intraperitoneal injections of oily solutions to mice.

When injections of both progynon B and naphthalene were administered, at least one day elapsed between the two injections.

Experiments on Tumor Formation in Males

Five groups with 25 or 26 mice in each were used in these experiments. Groups I and II were normal C3H males. Group I received weekly injections of 50 rat units of progynon B for 44 weeks, or until the appearance of a tumor. Group II received the same injections of progynon B and, in addition, a weekly injection of 0.05 cc. (2 mgm. or less) of 2,7-dihydroxynaphthalene. No castrated C3H males were used. Groups III, IV, and V were composed of line A albino males. Groups III and IV were normal. Group III received a weekly injection of 0.05 cc. naphthalene for 42 weeks in addition to the progynon B injection. Group V, composed of castrated albino males, likewise received weekly injections of both progynon B and naphthalene in the same amounts as the other groups. No castrated albino males received progynon B alone, since 30 castrated mice of this strain had been given such injections in a previous experiment (2).

Castration was performed when the mice were between 4 and 5 weeks of age, and injections were started in the week following the operation.

The results of the experiment are presented in Table I. There was no significant difference in tumor incidence nor in the age when tumors appeared in groups I and II, the normal C3H mice. Of those receiving progynon B alone, group I, 88 per cent developed tumors at an average age of 8 months, while among group II, those receiving naphthalene in addition to the hormone, 95 per cent of those living to tumor age developed tumors at the same average age. Of the 50 mice in these two groups, retention of urine to some degree, usually slight, was noted in 19, abscesses of the kidney were seen in 2, and scrotal hernia in 8. No hydronephrosis and no urinary calculi were observed. Scrotal hernia in C3H mice was not extreme, and did not interfere with normal function.

Of the 25 normal albino males in group III receiving progynon B only, one developed a tumor of the mammary gland at 10 months of age. Of the 26 normal albino males in group IV receiving 2,7-dihydroxynaphthalene in addition to progynon B, there was also a single mouse which developed a mammary tumor at 18.5 months of age. It will be noted from the table that a large number of mice in both these groups died before reaching cancer age. Scrotal hernia, usually bilateral, occurred in 26 of the 51 animals in these two groups, and was without doubt largely responsible for the high mortality. In 10 cases the bladder was greatly distended with retained urine, but no urinary calculi were observed. Hydronephrosis was seen in one case, and abscess of the kidney in 4.

Table I: Tumor Formation in Male Mice Receiving Progynon B and Progynon B Plus 2,7-Dihydroxynaphthalene

	Albino males				C3H males	
	Normal		Castrated		Normal	
	Progynon B	Progynon B + naphthalene	Prognynon B *	Progynon B + naphthalene	Progynon B	Progynon B + naphthalene
Number of mice	25	26	30	25	25	25
Number dying before tumor age	14	16	5	5	0	3
Number developing tumors	1	1	9	7	22	21
Per cent with tumors (of mice reaching cancer age)	9	10	36	35	88	95
Average age at development of cancer, months	18.5	10	12	10	8	8
Range in age, months	18.5	10	7.5–21.5	9–12	5–10	6–10
Average age at death, noncancerous, months	20	16.5	13.6	15	9	7.8

* Previous experiment (2).

Of the 26 castrated albino mice in group V that were given both progynon B and naphthalene, 7, or 35 per cent of those alive when the first tumor appeared in the group, developed tumors at an average age of 10 months. Retention of urine was noted in 15, hydronephrosis in 2, and abscess of the kidney in 2. No scrotal hernia occurred. The tumor incidence in this group approximated very closely that in the previously reported experiment (2), in which castrated males of this same strain were given progynon B only. Of 25 mice reaching cancer age in that experiment, 9, or 36 per cent, developed tumors at an average age of 12 months.

The high mortality among the normal albino males

so greatly reduced the number of mice reaching cancer age that percentages of tumors in these groups are meaningless. The type of scrotal hernia seen in these mice has been noted by previous workers in male mice receiving estrogen and has been described by Gardner (1). Gardner concluded that one factor in the formation of scrotal hernias seemed to be the presence of well developed testes, and the absence of hernias in castrate mice would appear to bear out this assumption. The lower incidence of hernias in normal C3H mice, however, remains unexplained, since the testes of these animals were well developed. Development of interpubic ligaments, the second factor noted by Gardner, was observed in males from both strains.

Comparison of castrate albino males of group V, receiving both progynon B and 2,7-dihydroxynaphthalene, and castrate males from the previously reported experiment in which progynon B alone was injected, showed no difference in the number of tumors induced. The average age at tumor development was somewhat lower in group V, 10 months as compared with 12 months in mice receiving progynon B alone. The range in age was also less in group V. No tumors developed as early and none as late as in the progynon B group.

This difference was not found, however, among the normal C3H mice of groups I and II, where the tumor incidence and age at development of tumors were nearly identical. The conclusion must be drawn, therefore, that the injection of 2,7-dihydroxynaphthalene does not influence the production of tumors in male mice by progynon B.

Effect of 2,7-Dihydroxynaphthalene on Growth of Male Mammary Glands

The mammary glands of castrated and normal albino and C3H males were studied to determine the effect of naphthalene on growth of the gland, and to discover whether differences in growth could be detected between the glands of mice receiving progynon B alone, and those receiving the hormone plus naphthalene. Whole mounts of mammary glands were prepared according to the method of Turner and Gomez (6). An enlarged image of the gland was then projected on drawing paper by means of a Promar apparatus, and silhouette drawings were made so that accurate comparisons between glands from different series of mice were possible.

Injections of 2 mgm. (or less) of 2,7-dihydroxynaphthalene alone were given subcutaneously at weekly intervals for several months to 20 normal males, 10 albino and 10 C3H. Duct growth resulted in the majority of cases. Among the albinos, however, the ducts were finer and growth was far more extensive than in the C3H males. In the latter the ducts were for the most part short and broad, with only a few fine branches at the tips.

A similar series of 20 castrated males was likewise studied. More extensive duct growth was observed in these mice, both in the albinos and the C3H, than in the corresponding normal group, although the increase in growth was more noteworthy in the C3H mice. Ducts in the glands from the albino males were again finer and longer than in those from the C3H.

Studies were made also of mammary glands from mice in groups I to V in the experiment on tumor production. These mice, it will be remembered, were: normal albinos, some of which had received only progynon B, and some progynon B and naphthalene; castrate albinos that had been given both hormone and naphthalene; and normal C3H mice, some of which had received only the hormone, and some both the hormone and naphthalene. These glands were taken at autopsy and consequently are not strictly comparable as to age or dosage of hormone and naphthalene. Within each strain, albino and C3H, however, the glands were closely similar in appearance. No consistent difference between the glands from mice receiving progynon B and those given progynon B plus naphthalene could be detected; but when the two strains were compared striking differences appeared between the glands from albino and C3H mice. Normal albinos, whether receiving progynon B only, or naphthalene in addition, showed extensive duct development with few alveoli. Castrate albinos that had received both hormone and naphthalene likewise had extensive duct development and attempts at interlobular duct formation, but few alveoli. On the other hand, all C3H mice exhibited complete lobule-alveolar development, and presented an entirely different picture from that of the albino mice.

It is apparent from these experiments that 2,7-dihydroxynaphthalene is capable of stimulating duct growth in male mice, but not of producing complete development of the gland with the formation of lobules and alveoli. Since stimulation of duct growth is an estrone effect, 2,7-dihydroxynaphthalene exhibits estrone-like properties in this respect.

The limitation of the effect of naphthalene to stimulation of duct growth may explain the failure of naphthalene-treated mice to show any difference in tumor age or incidence over progynon B treated animals, since tumor formation is always found subsequent to and associated with the formation and growth of alveoli. The difference in response of the glands to estrogen stimulation likewise provides an anatomical basis for the difference in tumor incidence in the albino and C3H strains.

Effects of Subcutaneous Injections of 2,7-Dihydroxynaphthalene on Female Mice

A study was made of the effects of subcutaneous injections of 2,7-dihydroxynaphthalene on female mice of the C3H strain.[1] Since the effects reported by Pincus followed intraperitoneal injections, and subcutaneous injections had been found by him to be less effective, it was necessary to discover what might be expected in female mice from different amounts of naphthalene injected in this manner for various periods. Both ovariectomized and normal females were used and observations were made of the effect of subcutaneous injections on the mammary glands, vaginal smears, and on ovary, uterus, and vagina. Studies were also made of the effect on the incidence of spontaneous tumors in bred females, and on the growth of spontaneous tumors after they had attained palpable size.

These experiments may be summarized as follows:—

Immature normal and ovariectomized females.—Eight females were ovariectomized at 6 weeks of age. Subcutaneous injections of 0.05 cc. (2 mgm. or less) naphthalene 3 times weekly were started 3 days later. Eight normal females of the same age were given similar injections, and 12 normal females of similar age served as uninjected controls. At 8 weeks of age all the controls had been in estrus once. Vaginal smears from the normal females receiving naphthalene injections were predominantly leucocytic through the 8th week, when some cornified and nucleated cells appeared. The first estrus was observed in this group shortly afterward, in the 9th week, but estrus cycles were extremely irregular. Vaginal smears from ovariectomized females receiving naphthalene were predominantly leucocytic through the 9th week when cornified and nucleated cells were present. Two had typical estrus smears during the 10th week, and such smears were observed to occur very irregularly in all females of this group. From these observations it would appear that 2,7-dihydroxynaphthalene either inhibits estrus or maintains diestrus in young normal females (which suggests a progestin effect). The occurrence of estrus in ovariectomized mice may indicate a direct estrogenic effect of the naphthalene. On the other hand, it may simply protect the estrogen formed in the adrenals of ovariectomized mice to a degree sufficient to induce irregular estrus. Hyperemia and enlargement of the uterus were noted in occasional treated mice of this series. Developing ova were found in the ovaries of normal females receiving naphthalene.

Effect on mammary glands.—Whole mounts of mammary glands from normal and ovariectomized, treated and control females were studied. The effects of the naphthalene were less obvious in females than in males. The duct system in treated ovariectomized females was often more extensive than in the controls, though alveoli did not develop earlier. In normal treated females there was possibly increased duct growth, but as the glands were very extensive in both treated and untreated females it was difficult to evaluate the differences.

Table II: Effect of 2,7-Dihydroxynaphthalene Treatment on Spontaneous Tumors

	Number of females	Number with cancer	Cancerous, per cent	Age at development of cancer, months	Number noncancerous	Age at death noncancerous, months
Controls	50	47	94	8.7	3	7.0
Naphthalene throughout breeding period	25	23	92	8.2	2	9.4
Bred females later treated with naphthalene	22	19	86	9.6	3	9.8

Effect of 2,7-Dihydroxynaphthalene on Tumor Incidence in Bred Females

2,7-Dihydroxynaphthalene was given to 25 C3H females from early maturity throughout the breeding period. For the first 11 weeks 0.05 cc. (2 mgm. or less) was injected subcutaneously once a week, and for the next 15 weeks the amount was increased to 0.1 cc. (4 mgm. or less). Breeding occurred normally and the females showed no effects from the injections. Two females died without cancer, one at 8 months and one at 10.5 months. Twenty-three females developed one or more tumors at ages ranging from 6.5 to 10 months. The average age at tumor onset was 8.2 months. These figures are summarized in Table II.

A second group of 22 females that had previously borne one or more litters was likewise given subcutaneous injections of 0.05 cc. naphthalene once a week until a tumor was observed or a mouse died. Nineteen of this group developed one or more tumors at an average age of 9.6 months. Three died noncancerous at an average age of 9.6 months (Table II).

A group of 50 control females from the regular breeding stock of the laboratory at that time had a tumor incidence of 94 per cent at an average age of 9.8 months (Table II).

[1] The author is indebted to Dr. Rosemary Murphy for much of the painstaking work and interpretation of the data involved in this portion of the study.

2,7-Dihydroxynaphthalene therefore did not affect breeding activity nor the formation of spontaneous tumors in normal female mice.

A group of 23 female mice that had already developed tumors was given weekly subcutaneous injections of 0.05 or 0.1 cc. naphthalene to discover if it would affect the growth of the tumor. Five died following the first injection. In all others the tumor grew progressively, and 7 developed one or more additional neoplasms during the period of treatment. 2,7-Dihydroxynaphthalene alone, therefore, did not inhibit the growth of tumors when injected after these had attained palpable size, nor did it prevent the development of multiple neoplasms.

CONCLUSIONS

1. Subcutaneous injections of 2,7-dihydroxynaphthalene failed to influence either the incidence or the age at appearance of progynon B induced mammary gland tumors in castrate line A albino or normal C3H male mice.

2. The high mortality from scrotal hernia among normal line A albino males precluded consideration of these groups.

3. Scrotal hernia did not develop in castrate albino males.

4. Scrotal hernias occurred less frequently and were less severe in normal C3H males than in normal albino males.

5. 2,7-Dihydroxynaphthalene stimulated duct growth in male mammary glands, but was not capable of producing complete lobule-alveolar development. This is considered to indicate estrone-like properties in 2,7-dihydroxynaphthalene.

6. Limitation of the effect of 2,7-dihydroxynaphthalene to the stimulation of mammary duct growth may explain the failure of naphthalene-treated mice to show any differences in tumor development from mice receiving progynon B alone.

7. The failure of line A albino males to produce numerous well developed mammary alveoli is suggested as an anatomical basis for their low tumor incidence.

8. 2,7-Dihydroxynaphthalene apparently either inhibits estrus or prolongs diestrus in young normal females. In ovariectomized females there was some evidence of either a direct estrogenic effect, or a protective effect on the estrogen formed in the adrenals sufficient to induce irregular estrus.

9. 2,7-Dihydroxynaphthalene did not affect breeding activity nor the formation of spontaneous tumors in normal female mice.

10. 2,7-Dihydroxynaphthalene injected subcutaneously into female mice with spontaneous neoplasms of palpable size did not inhibit the growth of such tumors nor the development of multiple tumors.

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The Effect of Sodium Pentobarbital, Paradichlorbenzene, Amyl Acetate, and Sovasol on Induced Resistance to a Transplanted Leukemia of the Rat*

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It was demonstrated in this laboratory that induced resistance to transplanted tumors in mice could be definitely modified by exposing the animals to x-ray or by repeated surface applications of coal tar (3, 4, 6). While both these agents are carcinogenic, it has not been established that their destructive action on resistance is dependent on or related to the cancer-producing property. In studies that are in progress at the present time, several toxic agents have been found which definitely increase the leukemia rate in a strain of mice having a natural tendency for the disease. The more active of these chemicals have been tested for their effect on induced resistance to a transplanted leukemia of rats.

MATERIAL AND METHODS

The transplanted lymphatic leukemia utilized in these experiments had its origin in a rat which had been injected in the groin with 1,2,5,6-dibenzanthracene. The characteristics of this strain have been described (5). During the period covered by the experiments to be reported, young rats of our stock, injected intraperitoneally with 0.2 cc. of leukemia cells, developed generalized leukemia in about 80 per cent of the animals inoculated.

The disease causes death in 7 to 12 days and is characterized by a high lymphocyte count with extensive involvement of the lymph nodes and thymus. Rats injected with 0.5 cc. of homologous living cells, either defibrinated blood or embryonic tissue, 12 days prior to the leukemia inoculation are about 85 per cent resistant to the inoculated disease (7).

The four materials selected for tests were sodium pentobarbital (veterinary nembutal), paradichlorbenzene (dichloricide), amyl acetate, and sovasol. For exposure to the volatile chemicals, the animals were placed in a wire basket suspended in a metal box with an airtight cover. Air, after bubbling through the volatile liquids, or a 5 inch column of crystals in the case of dichloricide, was kept flowing through the chamber during the period of exposure. A small electric fan mounted in the back wall insured an even distribution of the gas. In the case of nembutal, the solution was injected intraperitoneally.

EXPERIMENTAL PROCEDURE

For each individual experiment, about 30 young rats of approximately 55 gm. in weight were divided into three groups. The first group was given an intraperitoneal injection of 0.5 cc. of either defibrinated rat blood or hashed 15 day old rat embryo. The second received the same injection and 24 hours later was started on daily exposures to the substance under investigation. The third group, which received no treatment, served as controls for the activity of the inoculated material. Some 12 to 14 days after the tissue injections all the animals, including the untreated group, were inoculated intraperitoneally with 0.2 cc. of leukemic cells.

Effect of Sodium Pentobarbital on Induced Resistance

The commercial product known as veterinary nembutal was used in these tests. Young rats of approximately 60 gm. in weight would tolerate only 1.3 mgm. for the first 7 daily intraperitoneal injections, but by the 2nd week 2 mgm. caused no serious disturbance.

There were six individual experiments in this group, totalling 73 rats immunized and given from 14 to 19 intraperitoneal injections of nembutal, 90 rats immunized but no treatment given, and 61 rats which served as controls. In all the experiments there was a significantly higher percentage of takes in the nembutal-treated animals, but the degree of difference varied between experiments. The results of these experiments are given in Table I.

From these results, there seems little doubt that sodium pentobarbital has a suppressing effect on in-

* This investigation was aided by a fund for leukemia studies, contributed anonymously.

duced resistance to leukemia, but there is no immediate explanation of the great variability between experiments. It may be significant that the tests giving the most pronounced results were carried out in April and May, while those done between June and October showed less effect, and the least definite result was in an experiment carried out in late October. A similar variation in the action of this drug has been noted in another study, but this is not sufficiently clear-cut to suggest a seasonal factor until the tests are made through another cycle.

Effect of Paradichlorbenzene

Rats tolerated daily exposure to the fumes of paradichlorbenzene for 3½ hours, but this amount caused some disturbance to the general health. For the first tests, repeated exposures of one hour each were given, but for subsequent ones the time was increased to 2 and 3 hours.

The setup of this group of 6 experiments was the same as that described above. In the first 2 tests, where an hour's exposure was given, little effect on the induced resistance was noted, but in the 4 experiments where the time of exposure was increased to 2 and 3 hours a very definite influence was noted. As there was little variation between experiments, only the total figures are given in Table II.

Table II: Effect of Paradichlorbenzene on Induced Resistance

	Number of rats	Number positive	Per cent positive
Immunized and treated	40	27	67.5
Immunized, no treatment	39	8	20.5
Activity control	38	32	84.2

Effect of Sovasol

Sovasol, described as "a close-cut, highly purified naphtha," is used in industry as a solvent. Continuous exposure of rats to the fumes of this product for 15 minutes caused a high mortality. However, if the animals were given alternately 2 minutes in the gas and 1 minute in air a total of 14 minutes' exposure to the gas was tolerated through a series of daily exposures without bad effect. This dosage system was used in the following experiments.

The test consisted of 3 experiments, each made up of 3 groups of rats, one immunized and given from 15 to 17 exposures to the fumes of sovasol, one immunized with no treatment, and a third as control of the activity of the inoculated material. The combined results are given in Table III.

Effect of Amyl Acetate

Exposure to the fumes of amyl acetate for 2½ hours proved fatal for young rats, but repeated exposures for 40 to 90 minutes were tolerated without serious interference with the general health of the animals.

Table I

Experiment No.	Immunized and injected nembutal		Immunized, no treatment		Activity controls	
	Number	Per cent positive	Number	Per cent positive	Number	Per cent positive
15	10	80.0	9	11.1	10	80.0
16	20	80.0	20	30.0	10	80.0
18	8	62.5	10	0.0	10	80.0
19	17	41.2	20	0.0	10	80.0
20	7	42.8	10	10.0	10	70.0
21	11	27.0	11	18.1	11	81.8
Total	73	57.5	90	11.1	61	78.7

In the first 2 experiments of this group 19 exposures of 40 minutes each were given to the immunized rats with definite, but not striking, effects on resistance. In 3 subsequent experiments, where the length of the exposure was increased to from 60 to 90 minutes, the potential resistance was almost completely destroyed. The figures for the last 3 experiments are given in Table IV.

Table III: Effect of Sovasol on Induced Resistance

	Number of rats	Number positive	Per cent positive
Immunized and treated	49	36	73.5
Immunized, no treatment	29	8	27.6
Activity control	27	23	85.2

Table IV: Effect of Amyl Acetate on Induced Resistance

	Number of rats	Number positive	Per cent positive
Immunized and treated, 40 minute exposures	20	9	45.0
Immunized and treated, 60 to 90 minute exposures	30	21	70.0
Immunized, no treatment	50	9	18.0
Activity control	48	39	81.2

It is evident from the experiments described above that all the four substances tested have the property of reducing the potential induced resistance to inoculated leukemia. The relative effectiveness of the treatments is shown in Fig. 1. With the dosages used there was not complete destruction of the resistant state, but there is little doubt that with more frequent and prolonged treatments these potentially resistant animals could be rendered at least as susceptible to inoculation as the normal animal.

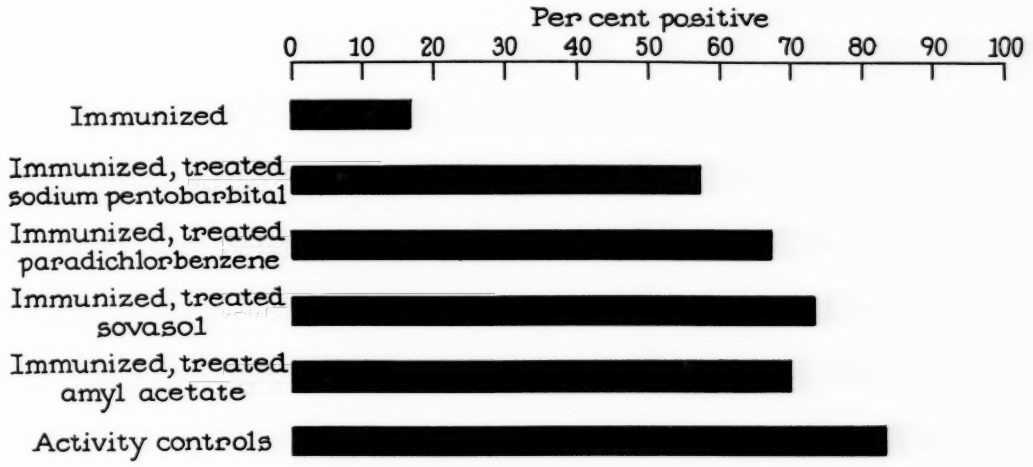

FIG. 1

DISCUSSION

It was noted some years ago that induced resistance to transplanted cancer may be definitely modified by exposing the animals to either of two carcinogenic agents, x-ray or coal tar. We have suggested, on the basis of some evidence, that induced resistance is a type of sensitization (2) and that agents interfering with the manifestations of the phenomenon act by suppression of the response of the animal (1). In the present study, four substances known to increase the leukemia rate in a strain of mice with the tendency to develop the disease have been shown to modify the induced resistance to a transplanted rat leukemia. If it could be established that such interference with induced resistance indicates that the suppressing agents have leukemia-inciting properties, it would materially simplify the test on the large number of toxic substances suspected of playing some part in causing the disease. If it can be shown that inciters suppress resistance it may indicate that they act, not by direct effect on the cells, but by the release of a natural tendency. An investigation to test these points is in progress.

SUMMARY

The repeated administration of any one of four toxic agents, sodium pentobarbital, paradichlorbenzene, amyl acetate, or sovasol, will definitely modify induced resistance to a transplanted leukemia in rats. The resistant state was induced by the injection of homologous living normal cells 12 to 14 days before the leukemia inoculation. Over 80 per cent of the rats receiving such treatment proved resistant to the transplanted leukemia, while only 16.5 per cent of the untreated rats were refractory. Potentially resistant rats exposed to the toxic agents showed a susceptibility to inoculation of from 57.5 per cent to 83.5 per cent, depending on the time and frequency of the exposures. It is noted that the four agents also have leukemia-inciting properties.

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The Skin Tunnel

A Device for Keeping Substances in Contact with the Skin

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In experiments designed to assess the importance of smegma and phimosis in the genesis of penile carcinoma, the following method of skin surgery was used: A buried skin tunnel was made and the substances to be tested were placed in this so as to be in contact with two opposing skin surfaces for a long, continuous period. This was done to imitate the condition which occurs when smegma is retained under the foreskin.

Mice about 4 weeks old were used. The skin tunnel was made on the back. The mouse was anesthetized with ether and the skin of the back shaved with a fine razor. Two parallel incisions, on either side of the midline, and about 1.5 cm. apart, were made with scissors through the skin from the pelvic to the scapular region. We then converted the central skin area into a tube by bringing its edges together with interrupted sutures of fine silk, using a small curved cutting needle and a small hemostat as a needle holder. Finally the outer skin edges were approximated over this with similar sutures so as to bury the tunnel completely. Sterile instruments were used, but no aseptic precautions were taken considering the well known resistance of mice. The operation can be completed in a few minutes. Hemorrhage was slight, for the only two vessels of importance, which are on the shoulder blades, can be avoided by not prolonging the incisions too far forward.

Healing was generally complete in 2 to 3 weeks. A probe then was passed through the tunnel. Our experimental material, which happened to be semisolid, was packed into the tunnel by inserting the nozzle of a small syringe, without a needle, into the caudad opening and forcing in the material. However, the technic for depositing material to be tested in the skin tunnel will naturally vary somewhat, according to the character of the experiment. It was not found practicable to close the cephalad opening, but this was made narrower by having the original incisions converge slightly toward their cephalad ends.

Advantages and Disadvantages of the Method

The pocket can be refilled repeatedly, and substances can thus be kept in contact with the same small skin area over long periods. The disadvantages are: (a) Growth of hair inside the pocket, which may prevent close contact. Attempts at making pockets out of the hairless skin of ear or tail were unsuccessful, and hairless mice are too delicate and too difficult to breed. (b) The pockets shrink considerably after completion, and continue to do so; this has been noted also with similar skin surgery in man. (c) If the substance used is infected, and there is any injury, the abscesses and ulcers which tend to form cause the pocket to slough off. (d) In spite of continued practice it has not been found possible to obtain 100 per cent of successes with a first operation; the average for 400 animals was 47 per cent, although in individual batches of from 6 to 10 animals 70, 80, and even an occasional 100 per cent has been achieved. A second operation was often successful when the first failed.

Possible Applications of the Method

The method, developed originally by surgeons to form a skin and muscle tunnel through which tendons or attachments of artificial limbs can be passed, is applicable to all carcinogens acting on the skin, and could be used for substances in pellet form and in weighed or measured doses. Radium, too, might be applied in this manner.

Metabolic Studies in Patients with Cancer of the Gastrointestinal Tract

XVII. The Conjugation of Phenols*

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INTRODUCTION

Hepatic dysfunction in patients with gastrointestinal cancer was found in this hospital to be a relatively frequent abnormality (1). Moreover, the functional derangement of the liver in most instances was associated with an altered chemical composition of the organ (2). Of 18 patients with cancer of the stomach, the livers of 16 had fatty infiltration and all probably had a decreased glycogen content.

One of the several tests used to ascertain the presence of hepatic insufficiency in the patients studied was their ability to synthesize and conjugate glucuronic acid. The formation of and the conjugation of glucuronic acid with aromatic compounds is apparently a function of the liver, and in the presence of hepatic parenchymal disease that function is considerably impaired (4). The daily excretion of abnormally small amounts of glucuronate in the urine was found in 44 of 50 patients with cancer of the gastrointestinal tract (1).

Much of the phenol and *p*-cresol excreted by the body is detoxified by conjugation with glucuronic acid (4). This conjugation, either with sulfuric or glucuronic acid, has been shown to take place primarily in the liver (6). The excretion of total phenols, on the other hand, appears to be a function of the diet ingested and the degree of intestinal fermentation (7). From the evidence it was believed that measurement of the urinary excretion of conjugated phenols should serve two purposes: It would (a) provide an index of hepatic function, and (b) perhaps explain the cause for a low urinary excretion of glucuronates by the patient with gastrointestinal cancer.

CLINICAL MATERIAL AND METHODS

The excretion of free and conjugated phenols by three groups of patients was measured. The first group consisted of 12 normal adults who were used as controls to determine the fraction of the total phenol excreted in the conjugated form. The second comprised 5 patients with hepatic cirrhosis who manifested both clinical and laboratory evidence of hepatic decompensation. The third was composed of 12 patients with cancer of the gastrointestinal tract; all these had a considerable degree of hepatic insufficiency as determined by several different hepatic function tests.

All the persons studied had taken normal diets. During the period of observation all excreted from 1 to 2 liters of urine per day. None received any phenolic compounds.

The free and conjugated phenols were measured in the urine by the method of Folin and Denis (3). The final color developed was read in a Pfaltz and Bauer fluorophotometer. The urinary content of glucuronates was determined by the technic of Maughan and his associates (5).

RESULTS

Normal persons.—The daily excretion of conjugated and total phenols, and that of glucuronate, was measured in the urine of 12 normal persons. Their urines were collected for periods of from 1 to 4 days. The urinary output of total phenol ranged from 228 to 807 mgm. and averaged 461.5 mgm. per day. Of these amounts, the conjugated phenol ranged from 24 to 43 per cent, and averaged 31.4 per cent (Table I).

The urinary excretion of glucuronate by these 12 subjects ranged from 308 to 800 mgm. and averaged 462.2 mgm. per day. These outputs of glucuronates are in agreement with those ascertained for another group of normal subjects in a previous investigation (1).

Patients with hepatic cirrhosis.—In contrast to the findings in normal persons were those obtained for the urinary output both of conjugated phenols and glucuronates by 5 patients with hepatic cirrhosis. The urines of these patients were collected for from 2 to 4 days. Although the daily excretion of total phenols was within normal limits (Table I), the

* The authors gratefully acknowledge a grant given by the National Cancer Institute.

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conjugated fraction of that substance was lower than that of the lowest normal value in 4 of the 5 instances, and lower than the normal average value in all. The excretion of total phenols ranged from 216 to 945 mgm. and averaged 516.4 mgm. per day, and the conjugated fraction ranged from 9 to 25 per cent of the total and averaged only 15.2 per cent. The urinary output of glucuronates, likewise, was in all instances abnormally low. These ranged from 118 to 186 mgm. and averaged 153.4 mgm. per day.

Patients with cancer of the gastrointestinal tract.— The phenols and glucuronate were measured in the urines of 12 patients with gastrointestinal cancer. The urine specimens were collected for from 1 to 4 days. Of the 12, 9 excreted normal total amounts of phenols, and 10 a normal conjugated fraction (Table I). The total phenol outputs ranged from 134 to 892 mgm. and averaged 422.1 mgm. per day; of these amounts from 19 to 42 per cent were conjugated.

It is interesting to note that although there appears to be no defect in the capacity of patients with gastrointestinal cancer to esterify phenol, their ability to synthesize or conjugate glucuronic acid is impaired. Among the 12 subjects studied, the daily excretion of glucuronates was abnormally low in 10. The daily outputs of this compound ranged from 75 to 336 mgm. and the average was 207.2 mgm., or less than one-half the normal value.

TABLE I: THE DAILY EXCRETION OF TOTAL AND CONJUGATED PHENOLS AND OF GLUCURONATES IN THE URINE OF NORMAL SUBJECTS, OF PATIENTS WITH HEPATIC CIRRHOSIS, AND OF PATIENTS WITH CANCER OF THE GASTROINTESTINAL TRACT

Subjects	Total phenols		Percentage of total phenols conjugated		Glucuronates	
	Range, mgm. per day	Average, mgm. per day	Range	Average	Range, mgm. per day	Average, mgm. per day
12 normal persons	228–807	461.5	24–43	31.4	308–800	462.2
5 patients with hepatic cirrhosis	216–945	516.4	9–25	15.2	118–186	153.0
12 patients with cancer of the gastrointestinal tract	134–892	422.1	19–42	30.2	75–336	207.25

DISCUSSION

The urinary excretion of total phenols apparently is influenced chiefly by the diet and the formation of those compounds in the gastrointestinal tract (7). Accordingly alterations in the output of total phenols by the groups of patients studied cannot be considered as significant, since no attempt was made to standardize their diets.

On the other hand, the esterification of phenols, chiefly with glucuronic and sulfuric acids, presumably occurs principally in the liver (6). When its cells are damaged, as in the livers of patients with decompensated hepatic cirrhosis, the ratio of phenol esters to total phenols would be expected to decrease. This expectation was confirmed by the abnormally low percentage values of conjugated phenols found in the urines of the 5 patients with hepatic cirrhosis included in the present study. Since glucuronic acid normally is used for the detoxication of phenols, it is possible that a limitation in the conjugation of those compounds might have limited the requirement for the synthesis of glucuronic acid. On the other hand, an *inability* of the liver to synthesize or conjugate glucuronic acid might have been the cause of a decreased detoxication of phenols by the patients studied.

The patients with cancer of the gastrointestinal tract have been shown to suffer from a considerable degree of hepatic dysfunction. This dysfunction is manifested in an impaired ability of their livers to fabricate prothrombin and albumin, to esterify cholesterol, to distribute properly vitamin A, to metabolize fat, to excrete bilirubin, to convert that pigment to urobilinogen, and to synthesize or conjugate glucuronates (1, 2). Despite this degree of hepatic insufficiency, it would appear from the present study that these patients still have a normal capacity to esterify phenols, a hepatic function notably decreased in the patients with decompensated cirrhosis of the liver who were studied here. The fact that the esterification of phenols does occur in most instances within normal limits, but that the excretion of glucuronates usually is abnormally low, indicates that other acids, probably sulfuric, now have taken over that function of glucuronic acid. To make this explanation more tenable, it would be necessary to demonstrate in these patients an increased urinary excretion of ethereal sulfates, determinations of which have not been made in the present investigation.

From the data at hand, then, it would appear that the impaired synthesis or conjugation of glucuronic acid by the liver of the patient with gastrointestinal cancer did not limit the capacity of the organ to conjugate phenols. Therefore, it is possible that the association of an abnormally decreased urinary excretion of glucuronates and an impaired ability to detoxify phenols by the patients with cirrhosis of the liver may have no causal relationship, and both might be due to damage of separate hepatic functions. Hepatic damage due to different causes will not always affect the same hepatic function. The fact that pa-

tients with cirrhosis of the liver no longer can conjugate phenols normally, but that patients with gastrointestinal cancer can do so despite the impairment of several other liver functions, may be another example of that phenomenon.

CONCLUSIONS

1. Patients with hepatic cirrhosis have an impaired ability to conjugate phenols. It is possible that the abnormally small amount of glucuronates excreted by these persons might be either the cause or the result of such impairment.

2. Patients with gastrointestinal cancer, who are known to have also a considerable degree of hepatic insufficiency, have a decreased capacity to synthesize or conjugate glucuronic acid. Nevertheless, their ability to esterify phenols remains unimpaired.

3. It is possible that the detoxification of phenols in patients with cancer of the gastrointestinal tract is not dependent necessarily upon the synthesis of glucuronic acid.

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Estrogen and 17-Ketosteroid Excretion in Patients with Breast Carcinoma*

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Investigations of the hormone content of the urine of cancer patients have been made repeatedly in the search for abnormalities that might reflect a disturbance in the endocrine physiology of the tumor-bearing person. Certain early reports suggested that excessive quantities of estrogens were excreted by cancer patients (8), especially women with genital tumors (32, 36, 50, 51, 56). Although these earlier observations on total estrogens have not proved correct, it has more recently been reported that special abnormalities occur in the relations of the different estrogen fractions to each other (44), particularly following the administration of estrone to women with uterine cancer (43).

The investigation of the urinary estrogens was begun in this laboratory eight years ago on patients with chronic cystic mastitis, a disease that seemed especially promising for this type of study on account of the associated menstrual abnormalities and other signs of endocrine disorder (62, 63). In the type of chronic mastitis termed adenofibrosis, the estrogen excretion patterns were found to be consistently normal; while in the type associated with nonpuerperal lactation, abnormalities in excretion were sometimes found, but these were compatible with the menstrual abnormalities also present and were not specific for the breast disease itself (63). Gynecomastia associated with cirrhosis of the liver has been studied by Glass, Edmondson, and Soll (25) with reported findings of low androgens and increased "free" estrogen in the urine. In breast cancer normal estrogen and androgen excretion has been found in both males (67) and females (46).

The plan of investigation adopted by us for a study of estrogen and androgen excretion in breast cancer included two parts: First it was necessary to compare the spontaneous excretion of estrogens and androgens of breast cancer patients with normal persons in comparable physiologic epochs. Secondly in a search for some peculiarity in the capacity of breast cancer patients to utilize, destroy, or eliminate estrogens it was determined to study the effects on urinary excretion rates of overloading the system with artificially administered hormone. These two methods of approach, designed to study the possibilities of somewhat different concepts of the relationship of the estrogens and androgens to breast cancer, form separate portions of the present communication.

METHODS

Hydrolysis.—Hydrolysis was carried out according to the method described by Callow and his group (2, 4), in which 40 cc. of concentrated hydrochloric acid is added to each liter of urine and the mixture boiled under a reflux condenser for one-half hour. A series of 20 urines run by us in duplicate to compare the Callow method with that recommended by Smith and Smith (54), the latter consisting of 10 minute boiling with 15 per cent hydrochloric acid by volume, failed to demonstrate any consistent difference in the yield of either the 17-ketosteroids or the estrogens.

Extraction.—The cooled hydrolyzed urine was extracted for 24 hours in a continuous ether extractor. The Kutscher-Steudel extraction apparatus for use with solvents lighter than water was used. Great efficiency was obtained by the employment of a sintered glass plate, through which the solvent was forced in minute bubbles, to afford maximum contact with the urine.

Separation of androgens from estrogens.—The ethereal solution was washed 2 or 3 times with saturated sodium bicarbonate and then extracted 5 times with 2 N sodium hydroxide. The neutral ethereal fraction was washed with water, filtered, and reduced to dryness on the steam bath and in the desiccator before determination of the androgens. The alkaline aqueous fraction was made acid to Congo paper with concentrated hydrochloric acid and extracted with ether. This ether extract was washed with water, filtered, and reduced to dryness, the residue being finally taken up in an amount of sesame oil suitable for the biologic assay of the estrogens.

In a previous investigation we had used benzene as the solvent for the extraction of the estrogens. In the belief that work with benzene constituted a definite hazard, ether was substituted. Recovery experiments, carried on during the course of the clinical studies, showed that estrogens and androgens were completely

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removed from urine by the ether. Although extraction of the phenolic estrogens from ether by sodium hydroxide has been a widely used method (64, 20, 46), we found toward the end of our clinical work that ether is not a satisfactory solvent at this stage, for some estrogenic activity remained behind even after 5 or 6 extractions with the alkali. The lower estrogen excretion rates here reported, as compared to figures noted in a previous study on chronic mastitis (63), may be attributed to this loss of estrogen into the neutral fraction. From a short trial with other solvents it now appears that, if benzene is not to be used, carbon tetrachloride is the best medium from which to separate the phenolic fraction by means of extraction with alkali.

Bioassay.—Bioassay of the extracts was carried out upon spayed adult white mice. These animals were standardized before each test by determining that they reacted with full estrus to a single priming dose of 2.5 gamma estrone given a week before their use. In the actual assay each mouse received one subcutaneous injection of 0.1 cc. of the unknown extract dissolved in sesame oil on each of 2 successive days, vaginal smears being made at 48, 72, and 96 hours after the first injection. Readings were made from a previously constructed dose response curve, based upon the injection of 300 mice with various amounts of crystalline estrone. Ten animals were injected for each final assay, and if from 30 to 70 per cent were positive the estrogen content was read directly from the curve. An average of 50 per cent of the animals in this laboratory have rather consistently shown full estrus when injected under these conditions with 0.1 gamma of estrone.

Fractionation of the estrogens.—The work of Pincus and Graubard (43) has suggested that peculiarities in estriol-estrone excretion ratios might characterize patients with cancer, particularly of the uterus. For this reason the original plans of this experiment called for a separation of the total estrogens of the urine into the estrone, estriol, and estradiol fractions. The procedure planned was the separation of the estriol as a strong phenol from the estrone and estradiol by means of weak alkali (5, 57) and the determination of the estradiol of the second fraction after inactivation of the estrone by semicarbazide. A complete separation of the "strong" from the "weak" phenols did not, however, prove successful in this laboratory. It was found, for example, that with the use of solutions of pure estradiol a minimum of 6 per cent was extracted by the weak alkali into the "estriol" fraction. This would not represent a great error if chemical determinations were depended upon, but such an error is enormously magnified by the bioassay. In our laboratory the amount of each estrogen required to produce estrus in the mouse was found to be as follows: 0.035 gamma of estradiol, 0.1 gamma of estrone, and 7.0 gamma of estriol. Dingemanse and Laqueur (9) noted a somewhat similar relationship. It is evident, then, that a trace of estradiol or estrone in the "estriol" fraction when assay is made with white mice will give greatly distorted estriol figures. For this reason the attempt to separate the three estrogens in single urine specimens was abandoned.

Assay of the androgens.—Androgens were determined according to the colorimetric method of Callow (1, 4), which had been adapted to reading on the Pulfrich photometer. This method is based on the Zimmermann reaction (68, 69), which is specific for all 17-ketosteroids, including substances not androgenically active. Bioassays with a slight modification of the Frank (15, 16, 35) baby chick comb method were also undertaken on a limited number of specimens. For a period of 7 days, 0.05 cc. of an oily solution was applied daily to the base of the combs of 1 day old, White Leghorn, female chicks in groups of 20. The ratio of body weight to comb weight was calculated for each chick and the biological content of a urinary extract was determined in terms of androsterone by reference to a standard curve.

TABLE I: ESTROGEN AND ANDROGEN EXCRETION IN YOUNG WOMEN WITH REGULAR MENSES

	Total estrogens			17-Ketosteroids by colorimetric determination			Androgens by bioassay		
	Number of patients	Number of tests	Daily average in gamma equivalents of estrone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone
Normal controls	3	22	3.4	3	31	9.4	3	8	2.1
Breast cancer	3	20	5.0	3	30	16.4	2	5	2.6

PART 1

SPONTANEOUS EXCRETION OF ESTROGENS AND 17-KETOSTEROIDS

The first part of the investigation consisted in the estimation of the total estrogen and total androgen excretion of patients with breast cancer, developing under four distinct physiologic conditions, each controlled by similar cases without breast cancer. The persons representing these four distinct physiologic conditions were the following: (a) young menstruating women; (b) young amenorrheic women; (c) women in the menopause; (d) men.

(*a*) *Young women with regular menses* (*Table I*).—Forty-two specimens, based on 24- or 72-hour urine

collections, were studied for total estrogens and a somewhat larger series for 17-ketosteroids. Since the daily estrogen excretion ranged from 0 to 34.0 gamma of estrone equivalents, the difference noted in average daily excretion between normal and cancer patients appears to be not significant. The excretion of 17-ketosteroids as well as of the biologically determined androgens was higher in the cancer groups, largely because of the very high figures of several specimens from a patient later found to have metastatic cancer of the adrenal.

(*b*) *Young women with amenorrhea* (*Table II*).—In terminal cases of breast cancer, as well as in other serious protracted illness, menstruation ceases and the excretion of estrogenic substances diminishes. The very low rate of 17-ketosteroid excretion is probably also a reflection of the general ill health and is not specific for cancer. Nevertheless, it is noteworthy that in these advanced cases with a maximum quantity of cancer tissue in the body there is certainly no elevation of 17-ketosteroid excretion. A pronounced fall in androgenically active material likewise appears to take place.

(*c*) *Women in the menopause* (*Table III*).—These showed little estrogen excretion but an average 17-ketosteroid excretion that did not differ from that of younger women. No difference existed between the normal women and those with cancer.

(*d*) *Males with breast disease* (*Table IV*).—These men showed no characteristic differences in daily excretion of estrogens. A relatively low excretion rate for 17-ketosteroids was noted both in cases of gynecomastia and in cancer of the male breast. Similar differences were present in the excretion rates of comb-stimulating substances. These observations suggest the possibility that gynecomastia and cancer of the male breast arise in the same type of person.

TABLE II: ESTROGEN AND ANDROGEN EXCRETION IN YOUNG WOMEN WITH AMENORRHEA

	Total estrogens			17-Ketosteroids by colorimetric determination			Androgens by bioassay		
	Number of patients	Number of tests	Daily average in gamma equivalents of estrone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone
Amenorrhea and tuberculosis	1	13	0.5	1	13	9.3	1	2	1.1
Amenorrhea and breast cancer	4	27	0.3	4	27	4.6	2	3	1.0

TABLE III: ESTROGEN AND ANDROGEN EXCRETION IN THE MENOPAUSE

	Total estrogens			17-Ketosteroids by colorimetric determination			Androgens by bioassay		
	Number of patients	Number of tests	Daily average in gamma equivalents of estrone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone
Normal control	2	13	0.4	2	16	8.1	2	5	1.2
Breast cancer	2	10	0.4	2	10	7.8			

TABLE IV: EXCRETION OF ESTROGENS AND ANDROGENS IN MALE BREAST DISEASE

	Total estrogens			17-Ketosteroids by colorimetric determination			Androgens by bioassay		
	Number of patients	Number of tests	Daily average in gamma equivalents of estrone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone	Number of patients	Number of tests	Daily average in milligram equivalents of androsterone
Normal males	2	7	0.7	5	19	21.2	1	4	4.4
Gynecomastia	10	10	0.9	10	10	11.9	1	1	2.4
Males with breast cancer	2	6	0.7	2	6	10.6	2	3	1.4

PART 2

EXCRETION OF ESTROGENS AND 17-KETOSTEROIDS AFTER HORMONE ADMINISTRATION

Although differences in the spontaneous excretion rates of total estrogens and 17-ketosteroids characteristic of breast cancer seem not to be present, it remains possible that these substances may be less efficiently handled by the cancer patient or the person susceptible to cancer development. Such a deficiency might be made apparent by burdening the organism with exces-

sive quantities of these substances artificially administered. Were a deficiency of this type present it might then manifest itself clinically by a susceptibility to disorders in the ovarian cycles, by unusual percentages of excretion of the injected hormone, or by disturbance in the excretion of other hormones.

Two women, one recently operated upon for breast cancer and a normal one of approximately the same age, submitted all urine excreted for seven successive menstrual cycles. The first cycle was devoted to the determination of the spontaneous excretion of estrogens and 17-ketosteroids. Thereafter two cycles each were assigned to a study of the effects of the injection of estrone, testosterone propionate, and progesterone respectively. Two cycles were devoted to each of the three hormones since it was known that the effects might be different depending upon whether administration was made before or after the time of ovulation. Care was taken to give the hormone injections on identical days of the cycle in the normal woman and cancer patient.

Clinical material.—Brief clinical histories of the two women follow:

M.P.—The normal one was a divorced woman of 37 with one child, 16 years of age, and a history of one spontaneous abortion 15 years prior to the study. There was no history of serious illness in the patient or record of cancer in the family. Menstruation, which had begun at the age of 11, occurred as a rule at 30 day intervals, lasted for 4 days, and was accompanied by slight dysmenorrhea. Breasts and pelvic organs were normal.

C.G.—The cancer patient was an unmarried girl of 27 with no history of previous serious illness or of cancer in her family. Menstruation occurred at 26 to 30 day intervals, lasted 3 days, was accompanied by moderate cramps, and preceded by some breast pain and swelling. A radical mastectomy for carcinoma of the breast with axillary metastases had been performed 4 months before these studies were undertaken. At the time of the tests there was no clinical evidence of cancer and the patient has remained well to the present time. The case was selected as one which might disclose physiologic peculiarities perhaps predisposing to cancer and not one on which to investigate disorders of steroid metabolism due to existent tumor tissue.

A comparison of the estrogen and 17-ketosteroid excretions and of cycle length in response to various hormone injections is shown for the two women in a series of seven charts. These are constructed to show average daily excretion of these substances as based on 72-hour urine collections. There are in addition two tables (Tables V and VI), which show whether the excretion rate of estrogens or 17-ketosteroids in the 9 or 10 day period during which injections were given was increased or decreased as compared with the same days of the cycle in the same patient before any injections had been started.

Control Periods before Hormone Administration

The total excretion of estrogen for a menstrual cycle has been reported to amount to from as low as 1,500 mouse units (14) to as high as 3,000 rat units (55), with many figures in between (6, 10, 18, 27, 51, 64, 66). Observations from this laboratory have previously shown normal monthly excretion rates of 3,000 to 6,000 international units. The monthly curve is known to show a peak of excretion at the time of ovulation, when as much as 800 international units or more may be excreted in a single day (26, 40). A second and less pronounced rise may develop shortly before menstruation.

The daily androgen excretion for normal women shows slight if any variations that can be correlated with the menstrual cycle. Werner (64) has recently reported normal 17-ketosteroid values for women from 5.4 to 19.6 mgm. daily, and Fraser and his associates (17) have reported an average of 9 mgm. daily.

In the control period the women of this study both exhibited a longer menstrual interval than they had reported as usual for themselves, the normal one having a 31 and the cancer patient a 34 day cycle (Fig. 1). The total estrogen excretion in both was lower than that previously found by us for normal women, the low figures being apparently a result of technical differences previously noted. Each showed typical peaks of estrogen excretion, the cancer patient exhibiting this rather late in her cycle. The 17-ketosteroid excretion averaged a little over 10 mgm. a day in her, a little below that in the normal control. All these figures lie within the normal range.

Administration of Estrone

The administration of estrogens to women has an effect on certain physiologic characteristics of menstruation as well as on the rates of steroid hormone excretion. Menstruation may be delayed for periods varying from 7 to 70 days, and ovulation prevented with subsequent elimination of dysmenorrhea at the next period (60).

When an estrogen is injected there is a rise in the estrogenic activity of the urine amounting, it is said, to only 6 per cent or less of the injected material (33, 34, 38, 45, 61). Since any special estrogen after its injection is converted at least in part to other forms of different biologic activity (65), bioassay of the urine without fractionation of the estrogens cannot give a figure that can be directly related to the amount of

injected material. In this report, then, percentages of recovery are only relative and used in comparing one case with the other.

in the cancer patient, to 45 days in the control. In each case a definite increase was found in the estrogenic activity of the urine, amounting in terms of biologic

TABLE V: EFFECT OF HORMONE INJECTION ON ESTROGEN EXCRETION

Time and type of hormone injections	Time of urine collection, days	Case	Estrogen excretion in gamma equivalents of estrone		
			Control cycle	Treatment cycle	Increase or decrease
ESTRONE: Pre-ovulation 80,000 units 9th to 15th days	7th to 18th	Normal	28.6	145.0	+116.4
		Cancer	26.3	111.8	+ 85.5
ESTRONE: Post-ovulation 80,000 units 18th to 24th days	17th to 26th	Normal	22.0	125.0	+103.0
		Cancer	85.5	105.0	+ 19.5
TESTOSTERONE PROPIONATE: Pre-ovulation 200 mgm. 8th to 14th days	8th to 16th	Normal	19.3	25.2	+ 5.9
		Cancer	20.4	18.8	— 1.6
TESTOSTERONE PROPIONATE: Post-ovulation 200 mgm. 15th to 21st days	14th to 22nd	Normal	24.9	87.0	+ 62.1
		Cancer	43.8	36.8	— 7.0
PROGESTERONE: Pre-ovulation 40 mgm. 8th to 14th days	8th to 16th	Normal	19.3	36.8	+ 17.5
		Cancer	20.4	48.5	+ 28.1
PROGESTERONE: Post-ovulation 40 mgm. 18th to 24th days	17th to 25th	Normal	22.0	24.4	+ 2.4
		Cancer	85.5	49.5	— 36.0

TABLE VI: EFFECT OF HORMONE INJECTION ON 17-KETOSTEROID EXCRETION

Time and type of hormone injections	Time of urine collection, days	Case	17-Ketosteroid excretion in milligrams		
			Control cycle	Treatment cycle	Increase or decrease
ESTRONE: Pre-ovulation 80,000 units 9th to 15th days	7th to 18th	Normal	108.6	87.6	—21.0
		Cancer	120.0	162.3	+42.3
ESTRONE: Post-ovulation 80,000 units 18th to 24th days	17th to 25th	Normal	84.1	65.1	—19.0
		Cancer	95.3	90.9	— 4.4
TESTOSTERONE PROPIONATE: Pre-ovulation 200 mgm. 8th to 14th days	8th to 16th	Normal	77.9	126.6	+48.7
		Cancer	91.0	156.6	+65.6
TESTOSTERONE PROPIONATE: Post-ovulation 200 mgm. 15th to 21st days	14th to 22nd	Normal	83.6	103.5	+19.9
		Cancer	94.2	117.0	+22.8
PROGESTERONE: Pre-ovulation 40 mgm. 8th to 14th days	8th to 16th	Normal	77.9	79.2	+ 1.3
		Cancer	91.0	77.4	—13.6
PROGESTERONE: Post-ovulation 40 mgm. 18th to 24th days	17th to 25th	Normal	84.1	55.8	—28.3
		Cancer	95.3	94.3	— 1.0

A decrease in the androgen content of the urine in women has also been reported to follow estrogen administration (28, 29).

Estrogens before ovulation (*Fig.* 2).—Estrone was first given in 4 doses of 20,000 international units from the 8th to the 15th day. Both women responded by a great prolongation of the menstrual cycle, to 38 days

estrogenic activity to about 1 per cent of the injected material. In each case a second peak in estrogen excretion occurred later in the month, perhaps representing the time of the delayed ovulation. A slight rise in the 17-ketosteroids was suggested in the cancer patient, but this was of doubtful significance.

Estrogens after ovulation (*Fig.* 3).—Estrone was

given during the 3rd month, in 4 doses of 20,000 international units each, from the 17th to the 24th day. Both women returned toward a shorter cycle type, and although the normal one menstruated on her 34th day and the cancer patient on the 26th day, these dates might be interpreted as within those on which spontaneous menstruation might have occurred.

The estrogen activity found in the urine during the periods of injection were identical in the two subjects. This represented a greater increase for the normal woman on account of the low values found in her control period. The 17-ketosteroid excretion was not significantly affected in either case by the estrone administration after the time of ovulation.

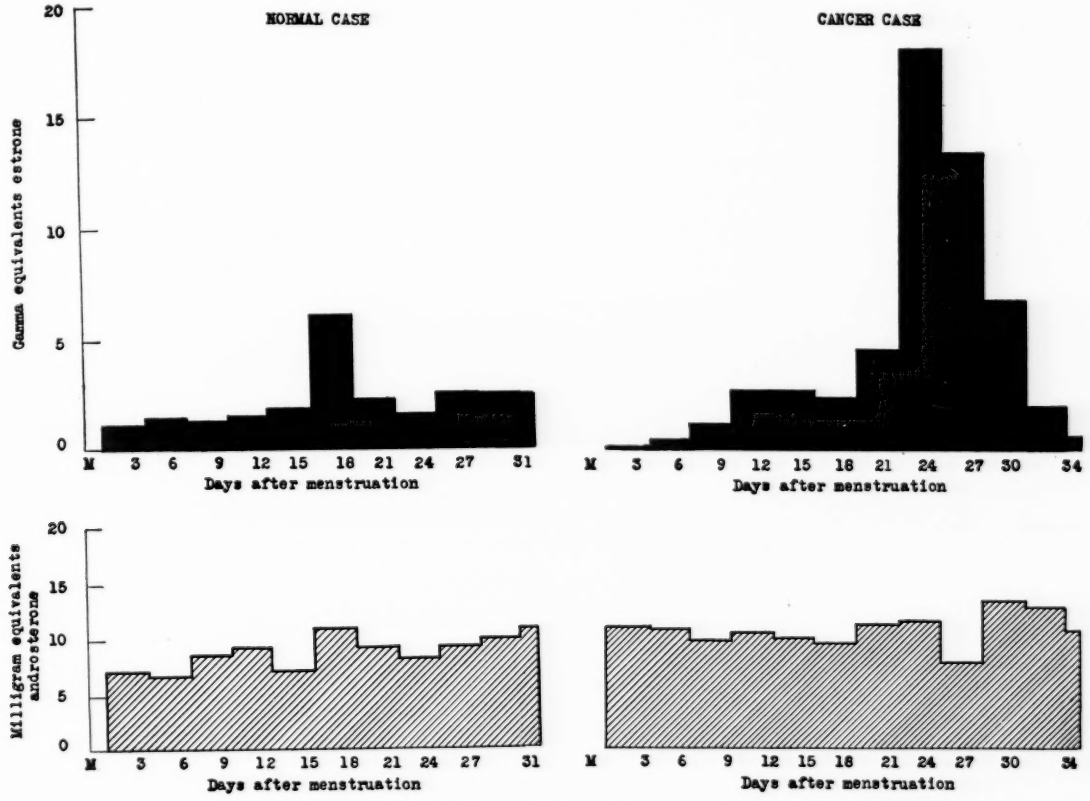
NORMAL CASE
CANCER CASE
Gamma equivalents estrone
Milligram equivalents androsterone
Days after menstruation
M 3 6 9 12 15 18 21 24 27 30 34

FIG. 1.—Excretion of estrogens and androgens. No treatment.

EFFECTS OF ADMINISTRATION OF TESTOSTERONE PROPIONATE

Testosterone in large doses has a profound effect on the function of the ovary and on the rhythm of menstruation. The action is said to be primarily an inhibition of the gonadotropic function of the anterior pituitary gland (22, 47). There follows as a result an inhibition of ovulation and corpus luteum formation with a delay in menstruation for longer or shorter periods of time (19, 21, 37, 41, 48). The critical dose, above which such effects occur, has been placed at 500 mgm. given during the pre-ovulatory phase (23). If it is given later in the month menstruation may occur, since ovulation has not been inhibited.

A rise in the urinary excretion rate of androgens occurs after the oral or intramuscular administration of testosterone propionate (13). The elevation of the subnormal values of male castrates and eunuchoids has been repeatedly observed (3, 11, 31). With small doses of testosterone propionate slight rises in androgen excretion have also been noted in female castrates (39).

A somewhat surprising observation has been that the administration of the male hormone causes a rise in estrogen excretion (3, 12, 31, 39, 58, 59). Various explanations have been offered, including the suggestion that the excreted estrogens were degradation products of testosterone or that the effect was due to certain androgens having some estrogenic qualities (7). It must be remembered that such increases in estrogen excretion rates have been observed chiefly in males and with moderate doses. With the administration to females of quantities sufficient to inhibit the activity of the anterior pituitary, and so prevent ovulation, a pronounced decrease in estrogen production and excretion may be expected.

Androgen administration before ovulation (*Fig. 4*).—Testosterone propionate was given in a total dose of 200 mgm., divided into 4 doses of 50 mgm. each, on

the 8th to 14th days. This relatively small amount was selected since it was hoped to study excretion rates without producing major physiologic distortions of the ovarian cycle.

With 200 mgm. given before ovulation the response of the two women was completely dissimilar. The normal subject, apparently missing an entire period, had period on the 30th, the cancer patient on her 21st day. A considerable increase in estrogen excretion occurred in the normal woman through the days of injection, while the cancer subject showed a slight but probably insignificant decrease.

The 17-ketosteroids of the urine also rose sharply in both cases during the period of administration but in

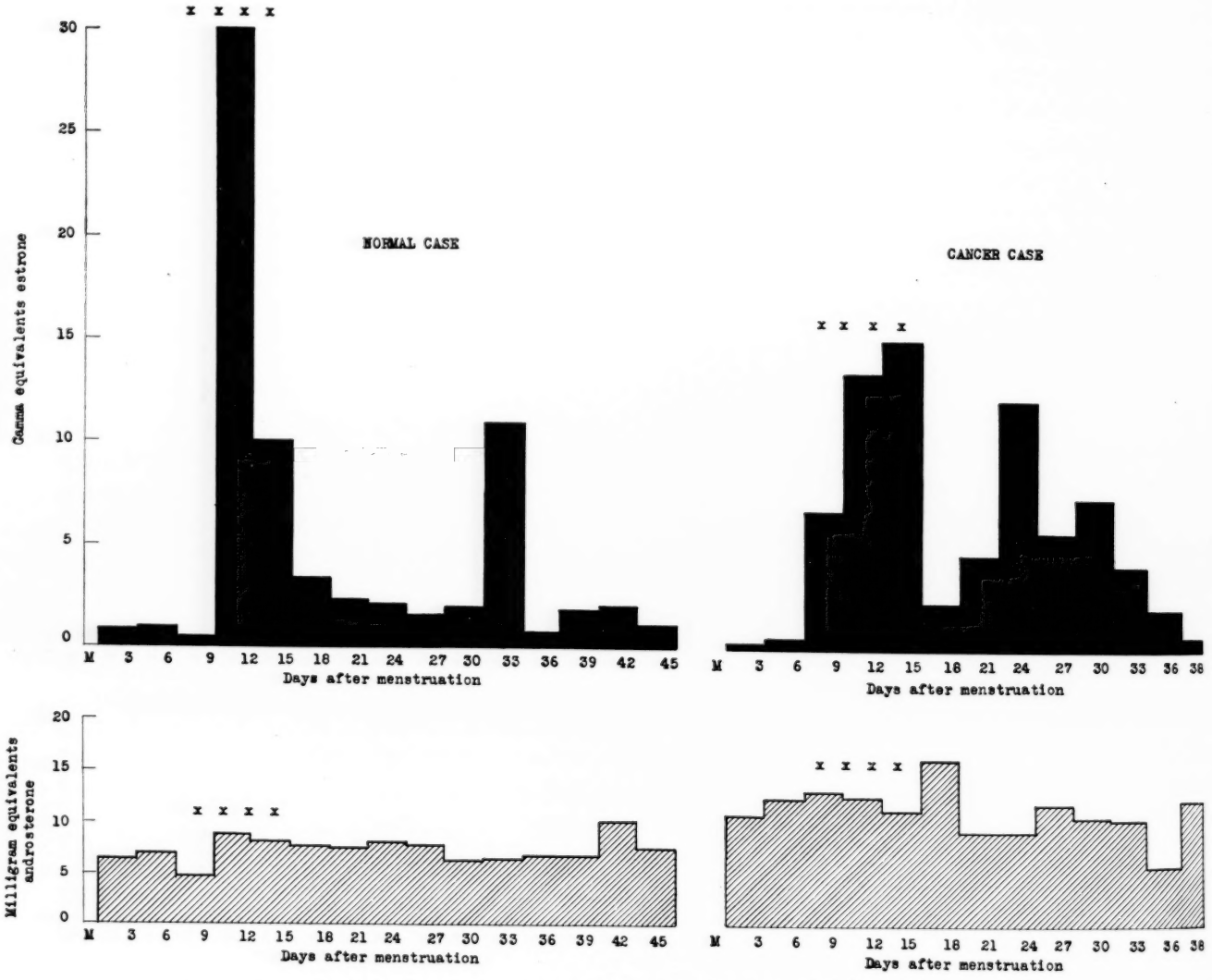
NORMAL CASE
CANCER CASE
Gamma equivalents estrone
Milligram equivalents androsterone
Days after menstruation
M 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45
M 3 6 9 12 15 18 21 24 27 30 33 36 38

FIG. 2.—Excretion of estrogens and androgens after estrone injections administered before ovulation. x = 2 mgm. of administered estrone.

no bleeding for a total of 59 days. The cancer patient began to menstruate 4 days after her last injection, or on the 18th day of her cycle. Both women experienced a rise in their androgen excretion rates, corresponding to a recovery of about 15 per cent of the injected testosterone propionate in each instance. The estrogen excretion was essentially unaffected in either case.

Androgen administration after ovulation (*Fig. 5*).—Testosterone propionate was given in the same dosage from the 15th through the 21st day. Differences in response were again noted, the control having her both to a less extent than when the injections were given before ovulation.

THE EFFECTS OF THE ADMINISTRATION OF PROGESTERONE

The effects of the injection of corpus luteum are apparently quite different from those which follow the administration of estrogens or androgens. When given before the time of ovulation in doses of 20 to 50 mgm., progesterone has been reported to produce almost im-

mediate uterine bleeding, which may be a "pseudomenstruation" (70, 71, 72) or a premature menstruation (24). Progesterone given at or after the time of ovulation is said to produce no effect on the menstrual cycle (71).

Progesterone has also been reported to increase the excretion of estrogens (42, 52, 53), although more recent investigation has indicated that progesterone has no effect on either estrogen or androgen excretion rates (49).

Progesterone before ovulation (*Fig. 6*).—Forty milligrams of progesterone was given in 4 divided doses, case was the probable date of the period affected, menstruation occurring on the 34th day in the control, on the 31st day in the cancer patient.

No increase in estrogen excretion was notable when progesterone was given at this time in the month, perhaps because a maximum corpus luteum effect was already present at this time. There was a decrease in rate of androgen excretion in the normal case.

DISCUSSION

The setting up of a controlled investigation of the urinary excretion of the sex steroids is enormously

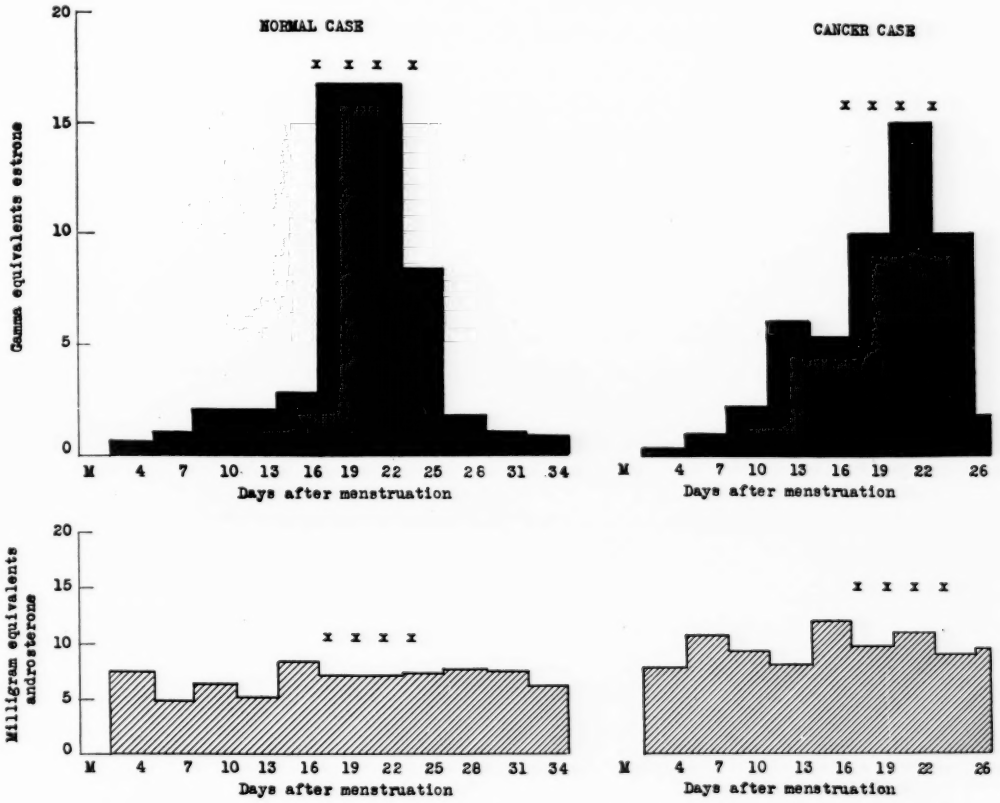
NORMAL CASE
CANCER CASE
Gamma equivalents estrone
Milligram equivalents androsterone
Days after menstruation
M 4 7 10 13 16 19 22 25 28 31 34
M 4 7 10 13 16 19 22 26
0 5 10 15 20

FIG. 3.—Excretion of estrogens and androgens after estrone injections administered after ovulation. x = 2 mgm. of injected estrone.

injected from the 8th to the 14th day. The two subjects again responded quite differently. The control showed no sign of bleeding until her 32nd day, when she went through what appeared to be a normal menstrual cycle. The cancer patient developed typical "intracyclic bleeding" or premature menstruation on her 18th day.

A considerable increase in estrogen excretion was noted during the days of progesterone administration when compared with the corresponding days of the control cycle in both cases. The effect of 17-ketosteroids was inconsistent and probably not significant.

Progesterone after ovulation (*Fig. 7*).—Forty milligrams of progesterone was given to each subject in 4 equal doses from the 18th to the 24th days. In neither

difficult, owing to the normal physiologic variations. This is especially true of the estrogen excretion in women, where the effects of age in relation to the menopause, and of ovulation and bleeding in relation to the menstrual cycle, produce variations from the average that must overshadow any probable specific change due to the presence of, or predisposition to, breast cancer. Similar, although much less extreme, variations may occur in the androgen excretion in the female and the excretion of both hormones in the male. It must be remembered furthermore that states of general health not directly concerned with the genital function may have a bearing on steroid excretion.

For any abnormality in steroid excretion rate to be

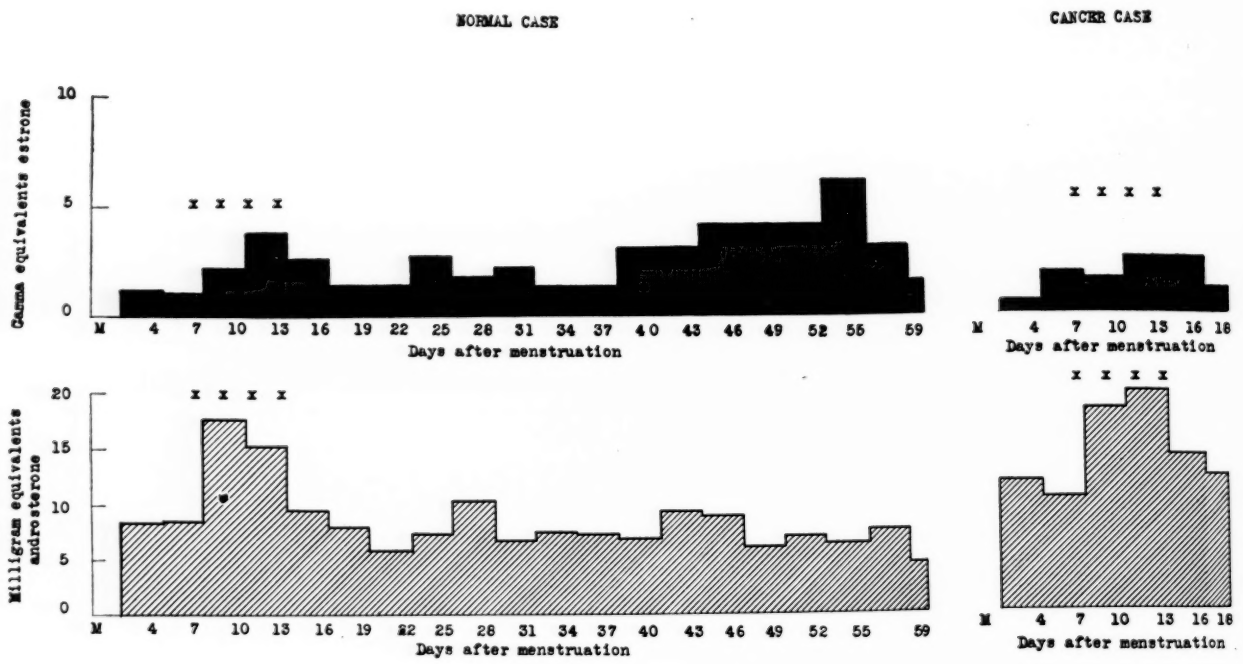
NORMAL CASE
CANCER CASE
Gamma equivalents estrone
Milligram equivalents androsterone
Days after menstruation
M 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 59
M 4 7 10 13 16 18

FIG. 4.—Excretion of estrogens and androgens after testosterone propionate injections administered before ovulation. x = 50 mgm. of testosterone propionate.

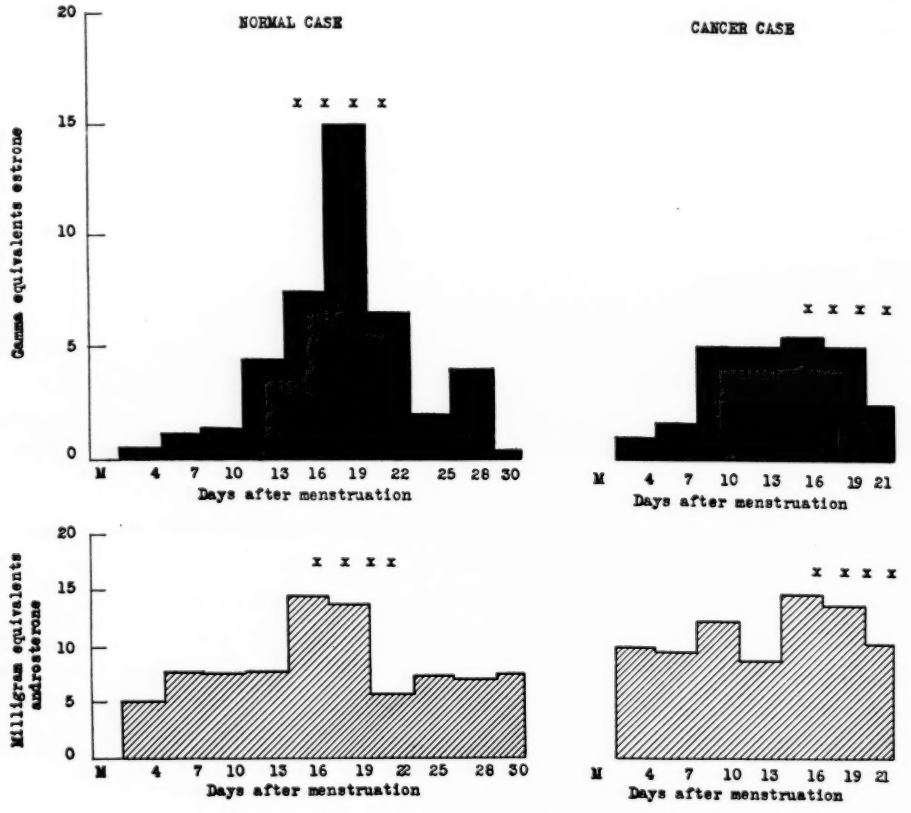
NORMAL CASE
CANCER CASE
Gamma equivalents estrone
Milligram equivalents androsterone
Days after menstruation
M 4 7 10 13 16 19 22 25 28 30
M 4 7 10 13 16 19 21

FIG. 5.—Excretion of estrogens and androgens after testosterone propionate injections administered after ovulation. x = 50 mgm. of testosterone propionate.

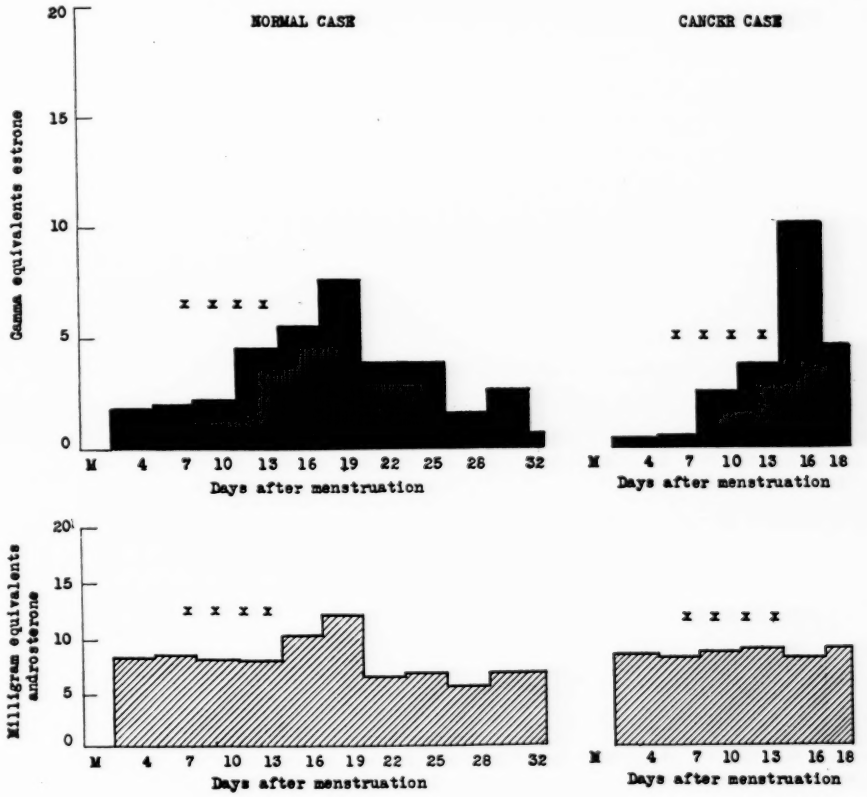
NORMAL CASE
CANCER CASE
Gamma equivalents estrone
Milligram equivalents androsterone
Days after menstruation
M 4 7 10 13 16 19 22 25 28 32
M 4 7 10 13 16 18

FIG. 6.—Excretion of estrogens and androgens after progesterone injections administered before ovulation. x = 10 mgm. of progesterone.

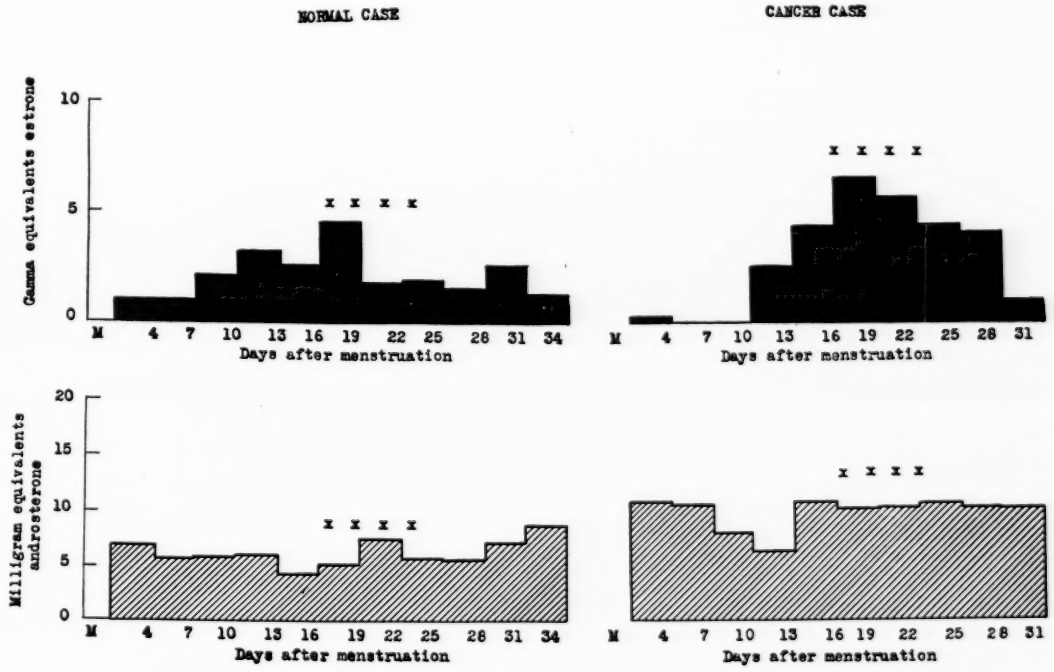
NORMAL CASE
CANCER CASE
Gamma equivalents estrone
Milligram equivalents androsterone
Days after menstruation
M 4 7 10 13 16 19 22 25 28 31 34
M 4 7 10 13 16 19 22 25 28 31

FIG. 7.—Excretion of estrogens and androgens after progesterone injections administered after ovulation. x = 10 mgm. of progesterone.

considered characteristic for some pathologic condition such as breast cancer, the deviation must be demonstrable against controls in as nearly identical physiologic states as possible; it must be consistent; and it must be of considerable magnitude. From the studies here presented and from a review of the literature it appears to us most unlikely that any measurable disturbance exists in the spontaneous excretion of total estrogens or total 17-ketosteroids in the presence of breast cancer.

In the second part of the investigation the attempt was made to expose some disturbance in steroid metabolism by observing the effects produced from the administration of excessive quantities of hormones to two women, one normal and one who had been treated for breast cancer. By the methods employed it was hoped that physiologic peculiarities in the patient susceptible to breast cancer might manifest itself in one of several ways; namely, in the excretion rates of administered hormones, in secondary effects on the excretion of other hormones, or in specific disturbance in menstrual function.

Although about 150 specimens were collected, and their content of estrogens and 17-ketosteroids determined, the investigation was confined to one cancer patient and one normal control. For this reason, the work cannot be considered in any sense final but rather exploratory to determine the most promising directions for further work.

The investigation led to certain observations of interest in the field of general sex hormone physiology as well as in that of cancer research. In particular the belief that the time of the cycle in which hormone injection is made will greatly influence not only cycle length but hormone excretion rate was amply confirmed.

With regard to cycle length, injection of neither estrone, testosterone propionate, nor progesterone after the time of ovulation seems to affect the date of the next menstrual period to any great extent. Given before ovulation, estrone postponed the onset of the next period, while with the other hormones inconsistent effects were produced. It seems possible that the determination of the threshold dosage of a given hormone required to produce certain characteristic disturbances of menstrual rhythm could be developed into a test of ovarian or anterior pituitary function.

The influence of hormone administration on urinary excretion rates was somewhat confused by the obscuring effects of the physiologic change in ovarian function resulting from the injection. A few points may be stressed.

Estrone administration caused a rise in the estrogenic activity of the urine regardless of the phase of the cycle in which it was given. The 17-ketosteroid excretion seemed little affected by estrone injections, although a slight rise may have occurred in the cancer patient when estrogen was given before ovulation.

When testosterone propionate was injected a considerable rise in 17-ketosteroid excretion was noted, but the percentage recovery in these menstruating women was not as high as that reported for similar experiments in males. The estrogen excretion when androgens were given before ovulation was reduced or unaffected. This may be explained on the basis of an indirect inhibition of the patient's own ovary by the androgens, which would more than compensate for any possible conversion of androgens to estrogenic material. After ovulation androgen injections were followed in one instance by a rise in estrogenic activity of the urine similar to that reported for males and female castrates.

Progesterone injections before ovulation were followed by a slight rise in estrogen excretion similar to that reported by Smith and Smith (52, 53). When they were given after ovulation no such rise occurred, perhaps because the administered progesterone had an insignificant physiologic effect in the presence of the patient's own actively functioning corpus luteum.

As for evidence of a functional predisposition to cancer nothing final can be said from this study. No striking contrasts in the rates of elimination of injected hormones were noted. Certain differences in hormone excretion were found, but these were not significant when only two cases were being compared. The response of the cycle length to the injections gave more definite contrasts, for the patient with cancer appeared consistently more susceptible to a shortening of the cycle than did the control.

CONCLUSIONS

1. The spontaneous excretion of total estrogens and 17-ketosteroids is not abnormal in breast cancer.

2. When estrone, testosterone propionate, and progesterone are injected into women, definite alterations occur in the rates of estrogen and 17-ketosteroid excretion in the urine and in the duration of the menstrual cycle. The exact alterations that occur depend to a considerable extent upon the time of the cycle in which the hormone is administered.

3. Certain contrasts in the response to hormone injection were noted in the seven month study of one woman with breast cancer and one normal control.

4. These differences in response undoubtedly reflect some physiologic differences in the two women and suggest clinical methods by which a physiologic predisposition to breast cancer may be sought for in further work.

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Further Studies of Specific Precipitin Antiserums for the Protein of Cancer Tissue. I*

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In a previous communication (4) we reported the results of studies on the preparation of specific antiserums for cancer tissue proteins. We have shown that such antiserums do not react with autolysates of the important normal tissues of the body but that they do react with the autolysate of the cancer tissue used in their preparation. The present report covers the results obtained in a much larger series of rabbits (139).

The method of preparation of these antigens is briefly as follows: Cancer tissue used must be free from normal tissue. Consequently, only a small percentage of the tumor is available for this work. The tissue is ground in a meat chopper, washed with physiological salt solution, and then ground in a ball mill for 48 hours with a small amount of physiological salt solution and toluol as a preservative. At the end of the grinding the tissue is strained through fine cheesecloth and only such as will pass through is used. This is washed in a high speed centrifuge with physiological salt solution until the washings no longer give a biuret reaction. The tissue is suspended in physiological salt solution and adsorbed on aluminum cream. This preparation is then injected intramuscularly into rabbits in small depots. As the lymph proteins cannot be removed quantitatively by the method of washing, blood protein precipitins appear in the rabbit serum and remain present until such time as the blood proteins are exhausted from these antigen depots (4, 7, 3, 1).

The rabbit serums were tested at monthly intervals with solutions of human albumin and human pseudoglobulin. The results show the time interval required for the disappearance of blood protein antibodies and the relative degree of activity of the antiserums for cancer proteins in 109 surviving rabbits injected with cancer tissue.

Rabbits were injected according to the method previously described (4) with cancer from the breast, cervix, colon, kidney, ovary, liver, rectum, stomach, and urinary bladder, fixed on aluminum cream. During the course of the work 30 of the animals died from accidents, respiratory infections, and other causes that had no bearing on the experiment itself.

The blood serum protein antibodies disappeared from approximately half (46 per cent) of the serums at the end of 6 months, whereas 54 per cent of the animal serums were free in 7 months, 85 per cent at the end of 8, and 88 per cent at the end of a 9 month interval. Blood serum protein antibodies persisted in 3 animals for a period of 10 months, in 2 for 11 and 12 months, respectively, and in one for 18 months. The precipitins for albumin disappeared at an earlier date than those for pseudoglobulin. The reason for this may be that the proteins of smaller particle size are exhausted from the aluminum cream depots more rapidly than those of larger particle size. Six of the animal serums never contained precipitins for blood serum proteins. Four animals did not develop any precipitins for either the blood serum proteins or the cancer proteins until after 3 months from the time of injection.

In previous work (4) we have shown that when a tissue is suspended in physiological salt solution under toluol and kept at body temperature the antigen slowly begins to appear and reaches a maximum after a number of hours. This amount of antigen is maintained for a period of hours, after which it begins to diminish and finally disappears from the autolysate. Autolysates were prepared by incubating some of the original tissue suspensions at 37° C. for 2, 4, 6, 8, 24, 32, 48, 56, 72, and 80 hours, respectively (4). These were consecutively numbered from 1 to 10. After the antibodies to blood serum proteins had disappeared the serums were titrated against autolysates of the respective tissues with which they were prepared. The precipitin tests were made by the contact or layer method. The reactivity of the serums was classified according to the number of the samples of autolysate with which they reacted: zero no reaction, one plus with 1 to 3 samples, two plus with 4 to 5, three plus with 6 to 7, four plus with 8 to 10 samples. The four plus serums were considered to be potent because they

* This investigation was aided by a grant from Eli Lilly and Company.

** Research Fellow in Surgery.

reacted positively with autolysates that contained relatively little antigen. These results are expressed in Table I.

TABLE I

Months after injection	Number of serums free of antibodies for blood proteins	Reaction of serums free of antibodies for blood proteins with autolysates of the tissue with which they were respectively prepared				
		0	+	++	+++	++++
2	1	1	0	0	0	0
3	12	4	3	0	3	2
4	17	12	5	1	0	2
5	32	20	4	6	2	1
6	50	32	6	3	2	1
7	59	45	7	1	3	1
8	93	78	5	2	5	3
9	96	82	0	8	4	2
10	104	86	9	4	2	0
11	106	100	2	3	0	1
12	108	102	3	2	0	1
13	108	104	3	1	0	0
14	108	106	2	0	0	0
15	108	108	0	0	0	0
18	109	108	0	0	0	1
19	109	108	0	0	0	1
20	109	108	0	0	0	1
21	109	108	0	1	0	0
22	109	109	0	0	0	0

Fifty-eight per cent of the serums had no precipitin antibodies for cancer tissue proteins after the precipitins for the blood serum proteins had disappeared. Only 11 per cent of the animals produced potent antiserums for the proteins of cancer. Fourteen per cent of the serums were poor (one plus), 13 per cent fair (two plus), and 4 per cent good (three plus).

The average time which the precipitins for cancer tissue proteins persisted was a little over 2 months after antibodies for the blood serum proteins could no longer be detected in the respective serums. The precipitins for the proteins of cancer tissue persisted from 1 to 8 months after these serums were free of antibodies to blood proteins. Occasionally antibodies for cancer tissue or blood proteins disappeared for a month, on the average, and reappeared the next month. Tables II and III each show 3 animals in which this occurred.

In 20 per cent of the animal serums the antibodies to blood proteins reappeared, but disappeared again on the average in 1 month. These results are expressed in Table IV.

DISCUSSION

Our results show that only a small percentage of the experimental animals develop potent antiserums for cancer. This percentage is definitely smaller than that obtained in other experiments with normal tissue proteins, such as kidney and liver. It may possibly be that the protein of cancer is less differentiated than that of the normal tissues and therefore less antigenic. The reappearance of blood protein and protein of cancer tissue precipitins after the serums have been previously free of them may be explained on the basis that some of the antigens stored in the normal tissues were liberated into the blood stream and called forth more antibody.

Considerable work has been carried out in an attempt to isolate in pure form the characteristic pro-

TABLE II: DISAPPEARANCE OF MALIGNANT TISSUE PROTEIN ANTIBODIES AND RECURRENCE

Animal No.	Months after injection											
	3	4	5	6	7	8	9	10	11	12	13	14
28			++++	++	0	+	+	+	0	0	0	0
68					++++	0	++++	0	0	0	0	0
161	+	++		++	0	++	+	0	0	0	0	0

TABLE III: DISAPPEARANCE AND RECURRENCE OF BLOOD SERUM PROTEIN ANTIBODIES

Animal No.	Titer to	Months after injection						
		3	4	5	6	7	8	9
9	Albumin	0	0	0	0	0	0	0
	Pseudoglobulin	+++	0	0	+++	+++	0	0
27	Albumin	++	0	0	0	0	0	0
	Pseudoglobulin	0	0	+	0	0	0	0
47	Albumin	++	0	0	0	0	0	0
	Pseudoglobulin	+	++	0	++	0	0	0

0 = no reaction.
+ = 1:100.
++ = 1:1,000.
+++ = 1:10,000.
++++ = 1:100,000.

TABLE IV: THE REAPPEARANCE OF ANTIBODIES TO BLOOD SERUM PROTEINS IN SERUMS WHICH WERE FREE OF THESE ANTIBODIES

Number of months after injection	4	5	6	7	8	9
Number of animals in which blood protein antibodies reappeared	3	1	5	8	0	5

tein of different tissues. So far our efforts have not been successful. If this can be accomplished, however, the preparation of specific antiserum will be much simplified and secondly, it will be possible to arrive at some reasonably accurate standardization of antiserum for the delicacy of reaction. The recent work of Claude (2), Shemin and Sproul (6), and Pollard (5) offers possibilities that may be of definite help in the isolation of cancer proteins.

SUMMARY AND CONCLUSIONS

1. Forty-two per cent (46 serums) of the serums of 109 rabbits injected with cancer tissue fixed on aluminum cream reacted with autolysates of cancer tissue with which they were prepared, but only 11 per cent (12 serums) were potent.

2. About half the animal serums were free of blood protein antibodies at the end of 6 months. The blood serum protein antibodies disappeared from the serums after from 2 to 18 months.

3. The precipitins for cancer tissue proteins persisted for a little more than 2 months after the serums were free of precipitins to blood serum proteins.

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Further Studies of Specific Precipitin Antiserums for the Protein of Cancer Tissue

II. The Application of in Vivo Absorption*

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When rabbits are injected with tissue proteins, precipitin antibodies for blood serum proteins appear in the serum in addition to precipitins for the tissue proteins (5, 6, 2, 1). There are three methods by which the former can be removed; namely, absorption *in vitro,* absorption *in vivo,* and allowing sufficient time to elapse following the injection for the exhaustion of blood proteins from the depots of tissue fixed on aluminum cream. Spinka and Weichselbaum (6) attempted to remove the blood serum protein precipitins from kidney and liver antiserums by absorption *in vitro,* using solutions of blood albumin and pseudoglobulin. They were able to rid the serum of the blood protein antibodies, but the tissue protein antibodies were also removed partially or entirely and invariably the potency of the tissue protein precipitins was definitely reduced. The absorption *in vitro* method failed to give us satisfactory results in similar experiments with cancer tissue. Hektoen and Welker (4) observed that the negative phase in precipitin production was specific. In rabbits immunized to many antigens, the injection of a single antigen resulted in the disappearance of the antibody for that antigen from the blood stream, with little or no effect on the titers of the rest of the antibodies. Spinka and Weichselbaum (6) were unsuccessful with the *in vivo* absorption technic in their work on precipitins for liver and kidney proteins. These investigators found that if sufficient time is permitted to elapse after the injection of tissue fixed on aluminum cream, the blood protein precipitins begin to drop in titer and finally disappear altogether. The presumption is that the blood proteins, being present in much smaller amounts than the tissue proteins, will disappear from the depots long before the tissue antigens become exhausted. This observation had been made previously by Hektoen and Welker (3) in their work on multiple antigens fixed on aluminum cream. When the serums of these animals no longer react with blood proteins, autolysates of the tissue can be used to determine whether the serum contains any precipitins for the protein of the tissue. By this method they were able to prepare specific antiserums for liver and kidney proteins. It seems desirable to reinvestigate the possible use of the *in vivo* absorption method in connection with the use of tissue antigens. If this method could be successfully applied in the preparation of serum without affecting the titer of the tissue protein precipitins many months could be saved in their preparation.

EXPERIMENTAL

Rabbits were injected with tissue from carcinoma of the human breast, colon, kidney, and stomach, and with normal human kidney fixed on aluminum cream. Two to three weeks after injection the animals were bled by cardiac puncture and the serums tested for antibodies for blood serum proteins. On the following day the animals that reacted with both albumin and pseudoglobulin were injected by way of the marginal ear vein with 5 cc. of a 1 per cent solution of each of these proteins in physiological salt solution. If they had antibodies to only pseudoglobulin, 5 cc. of a 1 per cent solution of this protein was injected. Blood samples were taken at 1, 2, and 24 hour intervals. The serums of the 1 and 2 hour samples were titrated against the blood proteins and also against autolysates (5) of tissue proteins. The 24 hour sample was titrated against blood serum proteins. The results on 10 animals are shown in Table I.

DISCUSSION

Antibodies to blood proteins could not be detected in the serums of 9 of the animals at the end of 1 and 2 hour intervals after intravenous injection of the proteins, but reappeared in 24 hours. In 5 of these animals antibodies for tissue proteins were present 1 and 2 hours after intravenous injection, whereas in 4 they could not be detected. In one animal it was

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impossible to remove the antibodies for blood serum proteins, even after a second injection of twice the quantities of albumin and pseudoglobulin.

While it is possible to apply the absorption *in vivo* method for the preparation of specific antiserums for cancer tissue with satisfactory results, the percentage of successful experiments is low. Many of the rabbits die as a result of intravenous injection of the antigens used for the removal of their antibodies, and in some we were unable to remove the antibodies for blood proteins by this method. Finally, the antibodies for tissue proteins may be absorbed as well as those for blood serum protein. In spite of all these difficulties, however, our results show that it is possible to prepare specific antiserums for tissue proteins in the course of a few weeks, as against 6 months or longer, by allowing sufficient time to elapse for the exhaustion of the blood proteins from the aluminum cream depots.

TABLE I

Animal No.	Tissue antigen fixed on aluminum cream	Titer of serum against blood proteins before intravenous injections: Albumin	Titer of serum against blood proteins before intravenous injections: Pseudoglobulin	Protein solution injected intravenously	Titer of serum against blood protein after *in vivo* absorption: 1 hour A	1 hour P	2 hours A	2 hours P	24 hours A	24 hours P	Reaction of serum with respective autolysates after intravenous injection: 1 hour	2 hours
329	Cancer of breast, No. 9	0	4	5 cc. 1% P	0	0	0	0	0	4	++	++
348	Cancer of kidney, No. 101	0	4	5 cc. 1% P	0	0	0	0	0	4	++++	++++
551	Cancer of breast, No. 111	0	1	5 cc. 1% P	0	0	0	0	Animal died		0	0
566	Cancer of breast, No. 111	0	4	5 cc. 1% P	0	0	0	0	0	4	0	0
570	Cancer of breast, No. 111	0	4	5 cc. 1% P	0	0	0	0	Animal died		0	0
572	Cancer of breast, No. 111	0	4	5 cc. 1% P	0	0	0	0	0	4	0	0
983 *	Normal human kidney	2	4	5 cc. 1% A								
				5 cc. 1% P	0	0	0	0	1	3	++++	++++
982	Normal human kidney	3	3	5 cc. 1% A								
				5 cc. 1% P	0	0	0	0	3	3	++++	++++
785	Cancer of stomach, No. 103	3	4	5 cc. 1% A								
				5 cc. 1% P	0	0	0	0	3	3	++	++
994	Cancer of colon, No. 102	3	4	5 + 10 cc. 1% A								
				5 + 10 cc. 1% P	3	3	3	3	3	3	?	?

Precipitin reaction to blood proteins:
A = Albumin.
P = Pseudoglobulin.
0 = No reaction.
1 = 1:100.
2 = 1:1,000.
3 = 1:10,000.
4 = 1:100,000.
* Experiment duplicated once in same animal.

Reaction of antiserums with autolysates:
++ = Reacted with 3 to 5 samples of autolysates.
++++ = Reacted with 8 to 10 samples of autolysates.

SUMMARY AND CONCLUSIONS

1. By the use of the absorption method *in vivo* we have been able to produce specific antiserums for tissue proteins. However, the percentage of serums obtained by the application of this method is relatively low.

2. This technic is valuable in that the time required to obtain a specific antiserum for tissue protein is reduced from several months to a few weeks.

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Heterotransplantation of Human Pseudomyxoma Peritonei into Mice and Rats*

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The problem of the absorption of grafted tissue is of theoretical as well as practical significance. As regards transplantation for clinical purposes, much thought has been given to the human ovary. According to Zondek and Wolff, both homotransplants and heterotransplants of fresh or preserved ovarian tissue of human origin are rapidly absorbed after implantation in man and the lower animals, and this process is accompanied by a simultaneous reduction of the hormonal effect on the organism of the host (Zondek). Examination of the site of transplantation and the hormonal reactions of the patients has revealed that ovarian transplants in women are completely absorbed in the course of one year (Zondek).

It seemed worthwhile, therefore, to find out whether ovarian tumors of human origin transferred to a foreign species behaved in the same way as normal ovary; *i.e.,* whether they were subject to absorption with the same degree of rapidity. To study this problem we implanted in animals a particularly active type of ovarian tissue, derived from a pseudomucinous cystadenoma ovarii.

In the literature we find reported a certain number of successful transplantations of malignant tumors from one species into another. But where these have been achieved in related animals, as for instance mice and rats (Roffo, Gheorghiu, Putnoky), hares and rabbits (Coca), chickens and pigeons or ducks (Murphy and Sturm), dogs and foxes (Sticker), frogs and other poikylothermic animals (Lucké and Schlumberger), they might be easily explained by the coincidence of two factors: the growth tendency of the inoculated material and the comparatively close relationship between the two species.

It is much more difficult to judge the outcome when heterotransplantation between far distant species is concerned; *e.g.,* man and the lower animals. Here two factors must be held responsible for the fate of the graft: the nature of the host tissue and the nature of the transplanted tissue.

The character of the host's tissue can be altered by blocking the reticuloendothelial system (Roskin), or by adapting the host to the tumor, as when Funk succeeded in transferring Ehrlich's mouse chondroma to rats by feeding it to them simultaneously with implantation.

We have transplanted human tumor material into 33 mice, 31 rats, 4 guinea pigs, and 16 rabbits, depositing it in the uterine cavity, Douglas' pouch, or the prevesical space of Retzius while the animals were simultaneously treated with prolan,[1] estrone,[1] or the blood of pregnancy (Table I). In none of our 84 cases, however, did the transplants show any growth tendency.

Tissues without definite protective reactions are said to be suitable sites for heterotransplantation. Such tissues are: chicken embryo (Murphy, Danchakoff), brain (Shirai, Oguchi, Yamasaki), testis (Gheorghiu), fluid of the anterior chamber of the eye (Keysser, Hegner, Smirnova, Nassetti, Greene).

Heterotransplantation may erroneously be considered successful when the stimulus exerted by decomposition products of the dying tumor ("necrohormones") elicits growth in the host's tissues (Lewin, Dagonet, Gargano, Keysser, Strauch, Nather, Domagk). Stieve, and Blumenthal as well, were able to enhance the formation of tumors in the host organism after heterotransplantation by using kieselguhr, and Keysser by sensitizing the heterotransplants with roentgen or radium rays.

The significance of the second factor, the nature of the transplant, is seen in Ehrlich's experiments with the zigzag transplantation of mouse carcinoma through rats-mice-rats, etc. Ehrlich, who in this way obtained as many as 14 passages, found that the tumors developed in the foreign organism only until their own resources in growth substances had been exhausted (about 1 week). The organism of a foreign species is unable to supply the necessary growth substances and possesses, therefore, some sort of immunity, which Ehrlich called athrepsia.

* Because of the difficulties of international communication the authors have not read proof of this article.

[1] We are indebted to Dr. Brent, Roche-Organon, Nutley, N. J., for his kindness in supplying us with pregnyl and menformon.

TABLE I: RESULTS OF TRANSPLANTING HUMAN MATERIAL INTO ANIMALS

Tissue	Quantity	Animals	Site	Outcome	Remarks
Wharton's jelly	2 pieces of 50 mgm. each	4 mice	Cavum Retzii and cavum Douglasi	Resorption after 1 week	2 mice injected daily with 1 cc. blood of pregnancy
	" "	4 rats	" "	" "	2 rats injected daily with 2 cc. blood of pregnancy
	" "	2 rabbits	Cavum Retzii and cavum uteri	Necrosis after 2 months	1 rabbit injected daily with 5 cc. blood of pregnancy
Hydatidiform mole	3 pieces of 100 mgm. each	4 mice	Pararenal	Resorption after 1 week	2 mice injected daily with 0.1 cc. blood of hydatidiform mole
	5 pieces of 100 mgm. each	4 rats	"	Resorption after 1 month	2 rats injected daily with 0.2 cc. blood of hydatidiform mole
	4 pieces of 200 mgm. each	4 rabbits	Pararenal and cavum uteri	Necrosis after 1 month	2 rabbits injected daily with 0.5 cc. blood of hydatidiform mole
Myoma with gelatinous degeneration	1 piece of 40 mgm.	13 mice	Pararenal	Resorption after 1 month	5 mice splenectomized
	" "	13 rats	"	" "	5 rats splenectomized
Serous peritoneal cyst	2 pieces of 30 mgm. each	4 mice	"	Necrosis after 2 weeks	
Mucous carcinoma of uterus	2 pieces of 20 mgm. each	8 mice	Cavum Retzii and cavum Douglasi	Necrosis after 2 months	4 mice injected with 2 × 2 r.u. prolan per week; 4 mice injected with 3 × 30 r.u. prolan per week
	2 pieces of 30 mgm. each	10 rats	" "	" "	5 rats injected with 2 × 2 r.u. prolan per week; 5 rats injected with 3 × 30 r.u. prolan per week
	3 pieces of 50 mgm. each	4 guinea pigs	Cavum Retzii and cavum uteri	" "	2 guinea pigs injected with 2 × 10 r.u. prolan per week; 2 guinea pigs injected with 2 × 10 i.u. estrone per week
	3 pieces of 200 mgm. each	10 rabbits	" "	Necrosis after 1–2 months	6 rabbits injected with 2 × 10 r.u. prolan per week; 2 rabbits injected with 3 × 50 r.u. prolan per week; 2 rabbits injected with 2 × 100 i.u. estrone per week
Pseudomyxoma peritonei (1 year in 4 per cent formol)	1 piece of 20 mgm.	12 mice	Pararenal	Resorption after 3 days	
	1 piece of 100 mgm.	4 mice	"	Resorption after 10 days	
	1 piece of 200 mgm.	" "	"	Resorption after 1 month	

When undertaking our experiments we made use of the work referred to in the foregoing paragraphs. According to clinical experience the human pseudomucinous cystadenoma ovarii displays a particularly vigorous local growth tendency, and since it may possibly be more embryonal in character than the majority of human tumors the prospects that heterotransplants would survive for a considerable length of time were rather good, as has been pointed out by Waterman. Furthermore, the use of such a gelatinous material permits the diffusion of nutritive substances from the adjacent tissues of the host into the graft, while, on the other hand, the intrusion of fibrous elements, and the resulting organization of the transplants, is made difficult. Hence the following experiments are particularly instructive in respect to the significance of the nature of the transplant.

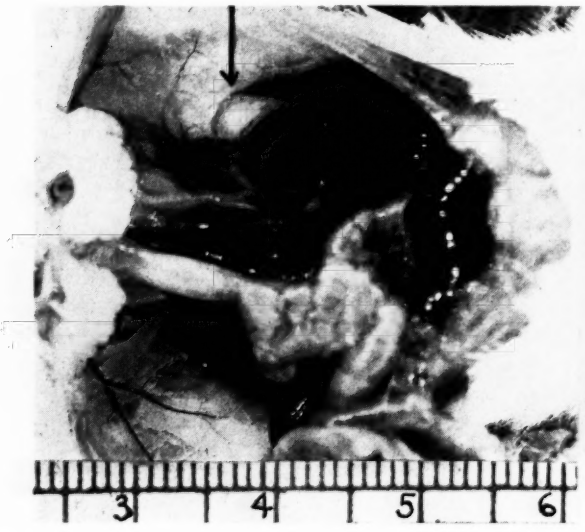

FIG. 1.—Graft of a human pseudomucinous cystadenoma after intraperitoneal implantation in a mouse (II 6). Three months after implantation just below the right kidney by the dorsal route the tumor had developed to about 5 times its original size. No necrosis.

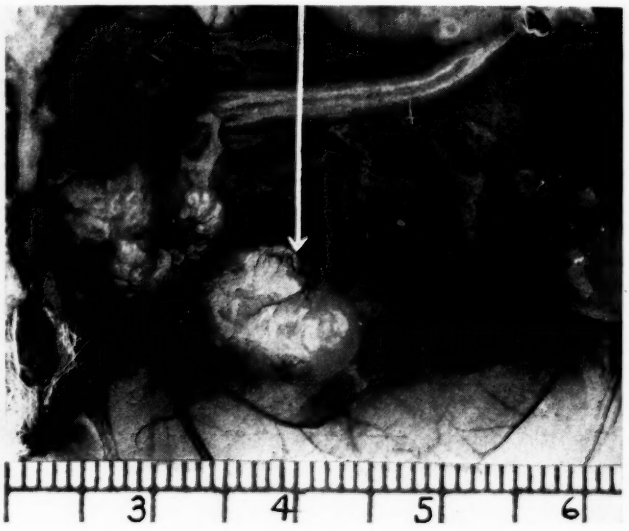

FIG. 2.—Graft of a human pseudomucinous cystadenoma after intraperitoneal implantation in a mouse (I 5). Three months after implantation just below the left kidney by the dorsal route the tumor had developed to about 20 times its original size. No necrosis.

The transplantation of pseudomucinous cystadenoma ovarii of human origin into the peritoneal cavity of rabbits has been carried out by Donat, Auché and Chavannaz, Toth, and Ahlström, all of whom found that absorption of the pseudomucin took place within a few weeks, the remaining portions being frequently encapsulated by granulation tissue. Lesions like those distinguishing pseudomucinous cystadenoma peritonei were not obtained (Naeslund).

EXPERIMENTAL

We implanted human pseudomucinous material into rats and mice, with far less success in the former than in the latter, and it soon became clear that intraperitoneal transplantation by the ventral approach produced in most cases only finely stalked or freely floating pseudomucinous cysts of the peritoneum with an inadequate blood supply in which, therefore, neither growth nor absorption took place. The most successful method was found to be intraperitoneal transplantation in mice by a dorsal approach. Here the grafts took in the majority of cases and underwent extensive proliferation in the neighborhood of the kidneys (Figs. 1 and 2).

MATERIAL

In the main we used the gelatinous material from a perforating pseudomucinous cystadenoma ovarii in a 54 year old woman. The patient, who was operated upon by the senior author, had a tumor of the right ovary as large as a man's head, with a number of peritoneal metastases. Pseudomucin was found in the peritoneal cavity, preponderantly in the subdiaphragmatic space and the cul-de-sac of Douglas. The total amount of material obtained from this patient consisted of about 5 kg. of primary neoplasm and about 10 of peritoneal tumor.

To the naked eye there was no difference between the two, both being composed of a whitish yellow, gelatinous material that yielded the usual reactions for pseudomucin.

Microscopically, however, the following difference was noted: The ovarian pseudomucin consisted of goblet-shaped columnar epithelial cells arranged in a

single layer and secreting pseudomucin (Fig. 3), whereas that from the peritoneum contained chiefly the mucinous substance itself with only a few cells (Fig. 4). Hematoxylin-eosin staining elicited a reddish blue color in the gelatinous substance, while with van Gieson's stain it assumed a yellowish green color and with Mallory's a light blue one. It should be said here that the peritoneal pseudomucin, with its small number of cells, grew much better upon intraperitoneal transplantation by the dorsal route than the ovarian type with its greater number.

In a group of control experiments we engrafted animals with other varieties of gelatinous material of human origin, transplanting Wharton's jelly, hydatidiform mole, myoma that had undergone gelatinous degeneration, serous cysts of the peritoneum, and mucous carcinoma of the uterus into 33 mice, 31 rats, 4 guinea pigs, and 16 rabbits. Furthermore, we placed the pseudomucinous material described above in 4 per cent formol (1:10) for one year. After the lapse of this time, the formol was removed by 4 hours' washing with water and the material grafted into 20 mice (Table I). The results were, in short, as follows: Heterotransplants of all the control material were absorbed and underwent partial necrosis. This took a few weeks, with larger transplants up to 2 months, as far as the living material was concerned (Table I).

A 20 mgm. piece of the pseudomucin that had been treated with formol (similar quantities had been used in the case of the living material) was absorbed within 3 days (12 mice), but larger fragments lasted correspondingly longer periods (100 mgm., 10 days; 200 mgm., 1 month). It thus appears that pseudomucin was deprived of its proliferative tendency by the formol, apparently because the few cells responsible for its growth had been destroyed.

FIG. 3.—Graft of pseudomucinous material of human origin. Part of the cyst wall with pseudomucinous masses. Mag. × 48.

FIG. 4.—Pseudomucin graft of human origin. Gelatinous material with almost no cells. Mag. × 48.

TECHNIC

Pieces of pseudomucin with an average weight of 10 to 20 mgm. were transplanted into a total of 15 adult rats and 37 adult mice of both sexes. After a certain time (1 week, 1 month, 2 months, 3 months) the animals were killed and the material obtained in each case was used for further transplantations after a sample had been reserved for microscopic examination. The majority of transplantations were carried out after 3 months.

The fixatives employed were formol or Zenker's fluid, the stains hematoxylin-eosin, Mallory's, or van Gieson's.[2]

3 of 8 cases (37 per cent), in mice pseudomyxomas were found in 4 of 8 cases (50 per cent). It was possible to transfer this material successfully to a second

TABLE II: RESULTS 3 MONTHS AFTER TRANSPLANTING PSEUDOMYXOMA OF THE HUMAN SUBJECT INTO RATS

First passage			Second passage		
Rat No.	Peritoneal implantation	Outcome	Rat No.	Peritoneal implantation	Outcome
I 1	20 mgm. dorsal	—			
I 2	20 mgm. ventral	—			
I 3	20 mgm. dorsal	50 mgm.	II 1	20 mgm. dorsal	—
I 4	20 mgm. ventral	—			
I 5	20 mgm. dorsal	500 mgm.	II 2	20 mgm. dorsal	Suppurating granuloma
			II 3	20 mgm. dorsal	—
I 6	20 mgm. ventral	200 mgm.	II 4	20 mgm. dorsal	—
			II 5	20 mgm. dorsal	—
I 7	20 mgm. dorsal	—			
I 8	20 mgm. ventral	—			

TABLE III: RESULTS 3 MONTHS AFTER TRANSPLANTING PSEUDOMYXOMA OF THE HUMAN SUBJECT INTO MICE *

First passage			Second passage			Third passage		
Mouse No.	Peritoneal implantation	Outcome	Mouse No.	Peritoneal implantation	Outcome	Mouse No.	Peritoneal implantation	Outcome
I 1	20 mgm. dorsal	200 mgm.	II 1	20 mgm. dorsal	—			
			II 2	20 mgm. ventral	—			
			II 3	20 mgm. dorsal	40 mgm.	III 1	11 mgm. dorsal	—
						III 2	" " "	—
			II 4	20 mgm. ventral	—			
			II 5	20 mgm. dorsal	—			
I 2	20 mgm. ventral	—						
I 3	20 mgm. dorsal	—						
I 4	20 mgm. ventral	—						
I 5	20 mgm. dorsal	400 mgm.	II 6	20 mgm. dorsal	100 mgm.			
			II 7	20 mgm. ventral	20 mgm.			
			II 8	20 mgm. dorsal	50 mgm.	III 3	20 mgm. dorsal	20 mgm.
						III 4	" " "	20 mgm.
			II 9	20 mgm. ventral	50 mgm.	III 5	10 mgm.	—
						III 6	" "	—
I 6	20 mgm. ventral	300 mgm.	Tissue culture	Explant rapidly developed	—	—	—	—
I 7	20 mgm. ventral	—						
I 8	20 mgm. dorsal	200 mgm.	II 10	20 mgm. dorsal	200 mgm.	III 7	10 mgm.	—
			II 11	20 mgm. ventral	—			
			II 12	10 mgm. dorsal	34 mgm.	III 8	10 mgm. dorsal	—
						III 9	" " "	—
			II 13	20 mgm. ventral	20 mgm.	Tissue culture	Explant poorly developed	—
			II 14	20 mgm. dorsal	20 mgm.	" "	" "	—

* This table contains for comparison some results of the explantation experiments, though these were not carried out in sufficient number to allow clear-cut results.

RESULTS

The results of our experiments are summarized in Table II (rats) and Table III (mice).

While in rats the transplantation of pseudomyxoma from a human source was successful in no more than

generation in 9 mice (56 per cent), in 6 of which (38 per cent) proliferation was vigorous enough to cause an increase in the weight of the inoculated material. Not before the third passage in mice did the graft remain stationary or undergo necrosis. In rats, on the other hand, not even a second passage was possible.

In the first mouse passage tumors were encountered that were 10 to 20 times as large and heavy as the original piece of pseudomucin implanted; *i.e.*, an increase from 20 mgm. to 200-400 mgm., or from the

[2] At our request, the Department of Experimental Pathology of this University undertook to grow explants of pseudomucin *in vitro* after the first and second transplantations into mice. Although various nutrient media were tried there was no sign of epithelial cell growth, the only proliferating cells being fibroblasts.

size of a lentil to that of a pea or bean. In the second there was at the most a tenfold increase; *i.e.,* from 20 to 200 mgm., or from lentil to pea size. The third passage no longer yielded any increase in size or weight, and even after the lapse of months the implanted material was still of its original size.

The implants invariably had the same appearance: grape-like, opaque, glassy formations with vascularization taking place from one pole, where they were covered with opaque, whitish yellow, proliferating fibrous tissue.

Histological examination of the transplants showed, in the first passage, a striking scarcity of cells and particularly little response on the part of the tissues of the host. A typical picture is given in Fig. 5, where three cysts of a multilocular pseudomucin transplant appear (mouse I6). Its weight was 300 mgm. after 3 months, and it was found in the neighborhood of the liver. The outer fibrous capsule was comparatively thin but slightly better developed than the thin fibrous septa. Organization of the pseudomucinous substance had begun especially in the small cysts which, to some extent, were invaded by migrating cells. In the larger cyst, apart from nuclei of epithelial origin there were some scattered nuclei of leucocytes. There were, moreover, eosinophilic inclusions within the pseudomucin that assumed a yellowish green color with van Gieson's stain and a red color with Mallory's, and therefore could not have been of fibrous origin.

In the second passage demarcation from the surrounding tissues was much more distinct. A strong fibrous capsule, from which migrating cells passed into the interior of the pseudomucinous mass, gave evidence of a beginning organization that clearly distinguished this tumor from those of the first passage, with their undisturbed growth (Fig. 6).

The unilocular pseudomucinous cyst that was found after 3 months in mouse II6 weighed 100 mgm. The fibrous capsule was better developed than that in the first passage (Fig. 5). Everywhere in the periphery there were leucocytes that had penetrated into the pseudomucinous body. The pseudomucinous mass which, in the first passage, had assumed a yellowish green color with van Gieson's stain, was now pink, indicating a beginning fibrous infiltration. With Mallory's stain the pseudomucin took a darker blue than did the original pseudomucinous mass.

In the third passage the pseudomyxoma transplants were completely demarcated from the surrounding tissues and there was no longer any growth. A greater number of septa and fibrous bands ran through the pseudomucinous mass and gave rise to migrating cells (Fig. 7).

The multilocular pseudomucinous cyst that was found in mouse III3 three months after transplantation, in the third passage, weighed 20 mgm. The fibrous capsule was comparatively thick, forming septa within the pseudomucinous mass interspersed with leucocytes. In the central region there was a villus-like formation consisting of fibrous tissue (van Gieson's or Mallory's stain).

DISCUSSION

Though we stated at the outset of this paper that the growth of gelatinous tissues on a foreign soil may be favored by the special nature of this tissue, no satisfactory explanation is afforded for the observation that it is particularly the pseudomyxoma peritonei which develops so well under these unfavorable conditions. During the last two years there had been no other case of this kind in our hospital, and thus we have had no opportunity to repeat our experiments. We are not certain, therefore, whether heterotransplantation would have been successful with every pseudomucinous cystadenoma ovarii. Moreover, it must be considered that, so far as growth capacity is concerned, the ovarian type may show a different behavior from that encountered in the appendix, since embryological characteristics are more evident in the former than in the latter, the genesis of which is still imperfectly understood (Naeslund).

It might be assumed that the comparatively weak stimulus exerted by grafted gelatinous tissue on its environment insures the undisturbed growth of such heterotransplants. Our experiments, however, showed that the gelatinous nature of the tissue alone is insufficient to explain the resistance of these heterotransplants in the body of the host. This is evident from the negative results of our experiments with Wharton's jelly, hydatidiform mole that had undergone gelatinous degeneration, peritoneal serous cysts, and mucous carcinoma, all of which were subject to early necrosis. Although our experiments testify to the extremely rare occurrence of pseudomucin-producing adenoma cells in the heterotransplants, it may be assumed that their excessive capacity to form pseudomucin—which is so much dreaded by the clinician—is the reason why heterotransplantation is so successful. This is equally suggested by the fact that in our experiments pseudomucin that had been denaturated by a 4 per cent formol solution (1:10) and in which the pseudomucin-producing cells had been thus destroyed, was completely absorbed 3 days after the implantation of 20 mgm. in mice.

It is difficult to understand why the few, scarcely recognizable pseudomucin cystadenoma cells should show such an extraordinary resistance towards the surrounding tissues of the host and still be able to produce pseudomucin in the heterotransplant. This is paralleled only by the excessive capacity of these cells to produce

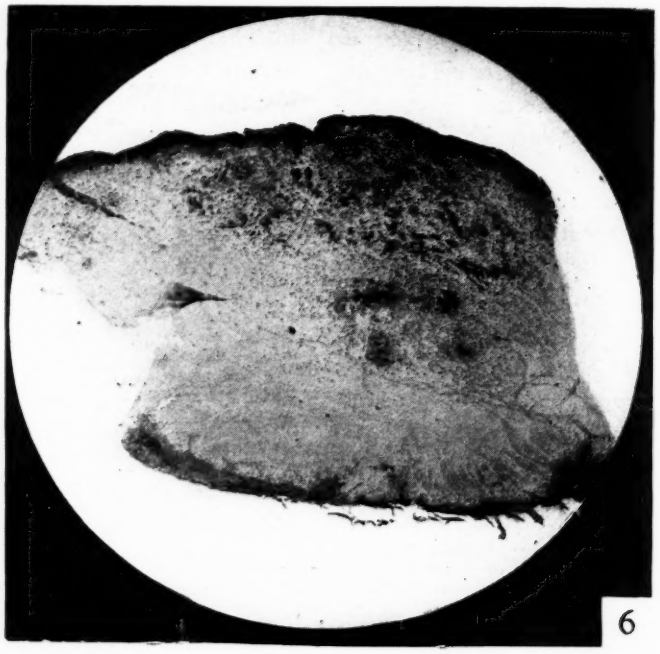

FIG. 5.—Graft of human pseudomucinous cystadenoma 3 months after implanation in a mouse (I6), first passage. The trilocular cyst was about 15 times as large as the original implant, contained few cells, and was only slightly organized. Mag. × 8.

FIG. 6.—Graft of human pseudomucinous cystadenoma material 3 months after implantation in mouse II 6, second passage. A portion of the cyst, which is about 5 times as large as the material originally implanted. Beginning organization, taking place from the periphery, interspersed with leucocytes. Mag. × 38.

FIG. 7.—Graft of human pseudomucinous cystadenoma material 3 months after implantation in a mouse (III 3), third passage. The implant has not grown. Advanced stage of organization through formation of fibrous septa. Mag. × 38.

pseudomucin in the human organism itself, where the pseudomucinous masses may cover the entire peritoneum. It is well known that even after every trace of these masses appears to have been removed recurrence may take place.

SUMMARY

Pseudomucinous cystadenoma ovarii in the human subject has been transplanted into mice, and with smaller success into rats also. Grafts showed definite growth after the lapse of 3 months provided the implantation was carried out intraperitoneally in the neighborhood of the kidney. In the second mouse passage these transplants grew less vigorously, and in the third not at all.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

ALLSOPP, C. B. [Strangeways Lab. and Univ. Dept. of Colloid Science, Cambridge, England] A WATER-SOLUBLE CARCINOGEN DERIVED FROM 3:4-BENZPYRENE. Nature, London, 150:548-549. 1942.

A solution of 3,4-benzpyrene in benzene and cyclohexane was irradiated in almost pure monochromatic ultraviolet light of 2,537 Å; the product, soluble in $M/100$ $NaHCO_3$, had produced at the time of writing squamous carcinoma of the skin in 4 out of 10 mice in 54 weeks. The active substance, which is unstable, has not yet been identified. Similar products were obtained from cholanthrene, 1,2,5,6-dibenzanthracene, phenanthrene, anthracene, and naphthalene.—E. L. K.

CRAMER, W., and STOWELL, R. E. [Barnard Free Skin and Cancer Hosp. and Washington Univ., St. Louis, Mo.] THE EARLY STAGES OF CARCINOGENESIS BY 20-METHYLCHOLANTHRENE IN THE SKIN OF THE MOUSE. I. EXPERIMENTAL TECHNIQUE AND MACROSCOPIC CHANGES. J. Nat. Cancer Inst., 2:369-378. 1942.

Preliminary to the study of early carcinogenesis it was necessary to establish a technic by which cancer of the skin could be produced: (1) in a reasonably short time, (2) in a high percentage of animals, and (3) in the center of the skin area subjected to treatment. With the technic that was adopted as standard, cancer could be induced in 100% of the mice, with low mortality and a latent period of 6 to 24 weeks. More than two-thirds of the cancers developed between the 12th and the 20th weeks and all occurred in the area of the skin to which the methylcholanthrene had been applied. Variations in technic showed that the size of the skin area exposed to the carcinogen was a greater factor in determining the response than either the concentration of the carcinogen, the frequency of its application, or the total amount of carcinogen administered.

The gross changes induced were: (1) epilation of the skin usually within 3 days; (2) dilatation of subcutaneous blood vessels, due both to the carcinogen and to the benzene solvent; (3) the appearance of shallow ulcerations, which healed; (4) the most important gross change—the appearance of swellings of 4 types, (a) elongated, ridge-like elevations that always regressed, (b) small, semi-globular, warty swellings that may or may not have been preceded by swellings of the first type, (c) benign tumors that sometimes arose from type b, and (d) malignant tumors. Types a and b are not true tumors. The nature of such swellings can be established only by histologic examination.—F. L. H.

CRAMER, W., and STOWELL, R. E. [Barnard Free Skin and Cancer Hosp. and Washington Univ., St. Louis, Mo.] THE EARLY STAGES OF CARCINOGENESIS BY 20-METHYLCHOLANTHRENE IN THE SKIN OF THE MOUSE. II. MICROSCOPIC TISSUE CHANGES. J. Nat. Cancer Inst., 2:379-402. 1942.

Studies were made of early changes taking place in the skin of 94 mice of the inbred Swiss strain, after cutaneous painting with a 0.6% solution of 20-methylcholanthrene in benzene according to a standard technic.

The effects of a single application are compared with those resulting from repeated doses and are considered as proceeding in three phases: (1) traumatization and repair; (2) excessive repair leading to epithelial hyperplasia; (3) development of skin cancer in a hyperplastic area.

Mice in each series showed decided differences in susceptibility to the action of the carcinogen.

Factors determining susceptibility and conditions determining carcinogenesis are discussed on the basis of the suggestion that the epithelial proliferation of the skin leading to the development of malignancy is elicited, not by the direct action of the carcinogen, which has a toxic inhibitory effect, but by a hypothetical substance formed in the skin in response to the carcinogen.

Four plates and 3 figures are shown.—F. L. H.

FISHMAN, M., SHEAR, M. J., FRIEDMAN, H. F., and STEWART, H. L. [Tumor Clinic, Beth Israel Hosp., Boston, Mass., and Nat. Cancer Inst., Bethesda, Md.] STUDIES IN CARCINOGENESIS. XVII. LOCAL EFFECT OF REPEATED APPLICATION OF 3,4-BENZPYRENE AND OF HUMAN SMEGMA TO THE VAGINA AND CERVIX OF MICE. J. Nat. Cancer Inst., 2:361-367. 1942.

Experiments testing the response of tissue lining the vaginal tract to low doses of a known carcinogen were carried out in a preliminary investigation of the hypothesis that smegma may be carcinogenic.

From 7 to 9 doses of 0.35 cc. of a suspension containing 2.5 gm. of smegma in 25 cc. of lard filtered at 38° C. were injected subcutaneously at intervals of 4 to 8 weeks into the left axillary region of twelve 3 months old mice of the A strain. Abscess formation and ulceration occurred, and the mice died 9 to 15 months after the beginning of the experiment. No tumors were found except 1 adenocarcinoma, which was probably spontaneous.

In 20 mice trauma was induced by inserting a metal plunger into the vagina 130 to 140 times during a 15 to 17 month period. No tumors resulted. Five mgm. of smegma were introduced into the distended vagina of 20 other mice

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2 or 3 times weekly for 12 months and less frequently thereafter. Five mice died during the 9th to 12th months after 72 to 115 applications. The rest died between the 14th and 16th months after 130 to 140 treatments. No gross evidence of tumor was found.

In a control experiment 5 mgm. of a 5% solution of 3,4-benzpyrene in cholesterol was introduced similarly into the vaginal canal of 10 mice 2 or 3 times a week. Two mice developed tumors during the 7th month, 6 more developed tumors by 10 months, and all mice had developed them by 14 months. The tumors appeared as papillary growths on the vaginal wall, and finally infiltrated the perivaginal tissues. Microscopically all were squamous cell carcinomas showing no involvement of the cervix. Each of 2 carcinomas was transplanted subcutaneously into 8 mice, but none of the transplants grew, possibly because of infection.

The authors believe that further investigation of the possible tumor-producing potency of smegma is warranted.—F. L. H.

LISLE, E. B. [Blackhill, County Durham, England] A COLOUR TEST FOR CERTAIN CARCINOGENIC HYDROCARBONS. J. Chem. Soc., 584. 1942.

Test papers are prepared in the dark by (1) soaking in aqueous solution of silver nitrate and methylene blue, (2) drying, and (3) soaking in aqueous solution of potassium bromide, and (4) drying. A drop of solution of hydrocarbon in toluene is applied and the paper is exposed to light of 4,200-4,400 Å for 5 minutes. Many carcinogenic hydrocarbons produce a blue-grey spot; non- or weakly carcinogenic hydrocarbons give a negative result.—E. L. K.

SHIMKIN, M. B., and LORENZ, E. [Nat. Cancer Inst., Bethesda, Md.] FACTORS INFLUENCING THE INDUCTION OF PULMONARY TUMORS IN STRAIN A MICE BY CARCINOGENIC HYDROCARBONS. J. Nat. Cancer Inst., 2:499-510. 1942.

The intravenous injection of methylcholanthrene dispersions in which the particles were 10μ to 20μ in size induced at least 10 times as many pulmonary tumors in strain A mice as similar dispersions in which the particles measured 1μ to 2μ. Apparently the neoplastic response depended upon the actual amount of the hydrocarbon that lodged in the lungs and not upon the amount injected into the organism. This indicates that the genesis of pulmonary tumors is the result of a local action of the hydrocarbon upon susceptible pulmonary tissue rather than a manifestation of a general systemic response to the chemical.

The lungs of young, small mice of strain A were more susceptible to the induction of pulmonary tumors with intravenous dibenzanthracene than the lungs of old, large mice of the same strain. The amount of hydrocarbon retained in the lungs was the same in both instances, but the smaller lungs of the young mice permitted a higher concentration of the hydrocarbon in the tissue.

The pulmonary tumor reaction was not influenced by foster nursing or by reticulo-endothelial blockade with trypan blue, and was the same whether the hydrocarbon was given in a single dose or in 5 repeated intravenous injections at 3 day intervals.—F. L. H.

STEINER, P. E., STEELE, R., and KOCH, F. C. [Univ. of Chicago, Chicago Ill.] THE POSSIBLE CARCINOGENICITY OF OVERCOOKED MEATS, HEATED CHOLESTEROL, ACROLEIN, AND HEATED SESAME OIL. Cancer Research, 3: 100-107. 1943.

Numerous extracts made from overfried and overbaked meats were tested for carcinogenicity in mice by subcutaneous injection and by feeding.

1. The fats from a mixture of beef, pork, and suet that had been overfried were tested by injection, by feeding, and by injection of the nonsaponifiable lipids.

2. The nonsaponifiable lipids from a second mixture of pork, beef, and suet prepared from the raw meat, after overfrying and after overbaking, were tested by injection.

3. The nonsaponifiable lipids from overfried beef were tested by injection.

4. The nonsaponifiable lipids from a mixture of beef, pork, and lamb were tested by injection.

Only one sarcoma was induced at the site of injection of these meat extracts. This occurred in a mouse injected with the benzene-soluble fats from overfried mixed meats.

Pure cholesterol heated to 200° C. and to 300° C. was not carcinogenic on subcutaneous injection into mice. Acrolein also was not carcinogenic. Sesame oil that had been heated to 350° C. was carcinogenic, inducing 3 sarcomas in 9 mice that lived for over 1 year.

These results, taken with those of other investigators, indicate that heated vegetable fats may be potentially more dangerous than the animal fats.—Authors' abstract.

SWAIN, G., and TODD, A. R. [Univ. of Manchester, Manchester, England] INDENO-2″:3″:2′:1′-BENZANTHRONE. J. Chem. Soc., 626. 1942.

This compound, and the 1″-keto derivative, were synthesized and were found by Haddow to have tumor inhibitory properties of a moderate order.—E. L. K.

TURNER, J. C., and MULLIKEN, B. [Coll. of Physicians and Surgeons and Presbyterian Hosp., New York, N. Y.] PRODUCTION OF SUBCUTANEOUS SARCOMA BY AZO DYE AND THE INFLUENCE THEREON OF LIVER FEEDING. Proc. Soc. Exper. Biol. & Med., 49:317-319. 1942.

Sixty young adult mice of the C strain received weekly 2.5 mgm. of o-aminoazotoluene dissolved in corn oil (mazola). Injections were made subcutaneously into the back, 0.1 cc. of oil being used for each dose. Forty animals were kept on Purina chow, while the remaining 20 received a supplement of dried fresh beef liver (20% by weight). After 11 months, 10 of the surviving mice were autopsied. All but 2 had developed subcutaneous sarcoma at the site of injection. Three of the 10 had been fed with liver and all 3 had sarcoma. While the feeding of liver gave protection against cirrhosis and cancer of the liver, it did not appear to influence the development of local sarcoma.—M. B.

WEIGERT, F. [Mount Vernon Hosp., Northwood, Middlesex, England] ABSORPTION SPECTRA OF 3-4 BENZPYRENE. Nature, London, 150:56. 1942.

Benzpyrene in the molecular state (*i.e.* in solution) exhibits a violet fluorescence. The fluorescence is green if the hydrocarbon is in the form of monoclinic crystals, blue for orthorhombic crystals, and yellow for colloidal suspensions. "The fluorescent spectrum of dissolved benzpyrene is unchanged in various solvents except for a slight

displacement of the entire group of bands." The absorption spectrum of the blue-fluorescing metastable orthorhombic modification of solid benzpyrene is entirely different from that of the dissolved hydrocarbon. (Cover slips upon which had been evaporated a benzene solution of benzpyrene provided thin crystal films of the hydrocarbon.)

The absorption spectra of the monoclinic crystal form and of the colloidal suspension can be studied only indirectly. A continuous spectrum is directed on a thin layer of the hydrocarbon so that there is little general absorption. Under these conditions the regions absorbing selectively show fluorescence and the absorption spectrum appears as fluorescent light ("fluorogram"). "The method permits of recording—at the present stage, qualitatively—the absorption spectra of systems which are not suitable for the usual spectroscopic methods."—I. H.

Hormones

GARDNER, W. U. [Yale Univ. Sch. of Med., New Haven, Conn.] TESTICULAR TUMORS IN MICE OF SEVERAL STRAINS RECEIVING TRIPHENYLETHYLENE. Cancer Research, 3:92-99. 1943.

Interstitial cell testicular tumors appeared in 7 of 13 mice of the JK strain that had received weekly subcutaneous injections of 5 mgm. of triphenylethylene for 250 days or more.

Seven of 17 mice of the A strain similarly treated had interstitial cell tumors, one of which has been transplanted for 4 generations. Although it has grown progressively only in mice that have received estrogens for 10 weeks, it has grown for 3 months after the last injection and has continued to grow after hypophysectomy.

One of 14 male C3H strain mice that received triphenylethylene died with a testicular tumor that had metastasized to lymph nodes, spleen, and lungs. Tumors of the testes were not found in mice of the C12I, N, or CBA strains similarly treated. The interstitial tissue was present in increased amounts in only 3 mice of these strains, where it consisted of large, clear, Leydig cells and "brown cells." Brownish cells and fibroblastic elements filled the small intercellular spaces in the other mice.

Hematopoietic foci occurred in the testicular tumors of all the JK mice and of 3 A mice. Some islands of hematopoietic tissues contained largely erythroblasts and normoblasts. Others showed developing granulocytes and megacaryocytes.—Author's abstract.

LIPSCHÜTZ, A. [National Health Service of the Republic of Chile, Santiago, Chile] EXPERIMENTAL FIBROIDS AND THE ANTIFIBROMATOGENIC ACTION OF STEROID HORMONES. J. A. M. A., 120:171-175. 1942.

Not only uterine but also extragenital subserous fibroids can be produced in the abdominal cavity of female guinea pigs by injection of estrogens. Fibroids were produced in the mesentery, spleen, pancreas, and abdominal wall. Under certain quantitative and timing conditions of treatment with estrogens, abdominal fibroids were almost always produced in the female guinea pig, seldom in the male, and not at all in the rat. All estrogens, natural and artificial, free and esterified, were shown to be tumor-inducing, even in very small doses. This tumor-inducing action is largely inhibited by the administration of estradiol benzoate or testosterone propionate, and especially by desoxycorticosterone. It cannot be predicted whether the same results will be obtained in women, because of the profound influence of species on the experimental results, but the method of inhibition awaits trial in the hands of the gynecologist.—H. G. W.

Radiation

APPERLY, F. L., and CARY, M. K. [Med. Coll. of Virginia, Richmond, Va.] THE DETERRENT EFFECT OF LIGHT UPON THE INCIDENCE OF SPONTANEOUS BREAST CANCER IN STRAIN A MICE. Brit. J. Exper. Path., 23: 133-135. 1942.

Strain A mice were exposed to light from a G.E. model F ultraviolet lamp for varying periods. In the small numbers used, the cancer rate in the breeding females was less than in the controls (41% as compared with 80%).

(Note: References to *Pierce* in text and bibliography should read *Pearce*).—A. H.

KIRBY-SMITH, J. S., BLUM, H. F., and GRADY, H. G. [Nat. Cancer Inst., Bethesda, Md.] PENETRATION OF ULTRAVIOLET RADIATION INTO SKIN, AS A FACTOR IN CARCINOGENESIS. J. Nat. Cancer Inst., 2:403-412. 1942.

The paper presents the results of comparative studies on the penetration of ultraviolet radiation into human and mouse skin and the relation of these findings to carcinogenesis. The method consisted in illumination of the epidermis with a collimated beam of monochromatic light from a mercury arc source, and photoelectric estimation of the emerging radiation. Two methods of measuring the emerging radiation are given in detail.

Observations on human (cleared) and mouse epidermis led to the conclusion that the absorption spectra in both cases are of the same general character in the region of 3,000 Å, and that absorption in this region is principally due to protein. On the other hand, uncleared human epidermis transmitted wave lengths shorter than 3,200 Å (the region with tumor-inducing properties) less extensively than did mouse epidermis. The transmission of human epidermis was shown to vary considerably between individuals, and for different parts of the body.

Although mouse epidermis, repeatedly irradiated and thus comparable to well tanned human skin, showed greatly reduced transmission, both irradiated and normal mouse epidermis transmitted more radiation from the tumor-inducing region than any of the samples of human epidermis. These differences in penetration of radiation into mouse and human skin, respectively, are in harmony with the finding that tumors of dermal origin predominate in mice subjected to ultraviolet radiation, while virtually all human cutaneous tumors of exposed areas are epidermal in origin.—F. L. H.

OUGHTERSON, A. W., PLAUT, J., and LAWRENCE, E. A. [Yale Univ. Sch. of Med., New Haven, Conn.] THE TUMOR RESPONSE TO ROENTGEN IRRADIATION AS INFLUENCED BY THE HOST-TUMOR RELATION. Am. J. Roentgenol., 47:207-209. 1942.

The radiocurability of a transplantable mammary carcinoma in strain A mice was determined as 1% for doses

of 2,500 r and 48% for doses of 5,000 r. The same tumor could be transplanted readily into the F_2 generation of a cross between strain A and strain C57. However, the tumors in these F_2 mice were cured in 82% of the cases by 2,500 r and in 97% by 5,000 r. Even after allowance is made for the spontaneous tumor regressions, which were observed in 28% of these F_2 mice, the radiocurability of the tumors was much greater in the F_2 hosts. Attempts to affect the radiocurability of mouse tumors by altering the diet of the host or by foster nursing yielded inconclusive results. It is suggested that roentgen irradiation acts to augment natural host resistance to tumor growth.—C. E. D.

Biochemistry and Nutrition

CHITRE, R. G., and KHANOLKAR, R. V. [Tata Memorial Hosp. for the Treatment of Cancer and Allied Diseases, Bombay, India] STUDIES IN ESTERASE (BUTYRIC) ACTIVITY. II. ESTERASE CONTENT OF LIVERS OF MICE AND ITS EXCRETION IN STRAINS SUSCEPTIBLE OR INSUSCEPTIBLE TO MAMMARY CANCER. Cancer Research. 3:88-91. 1943.

In a previous paper (Cancer Research, **2**: 567-570. 1942) it was demonstrated that esterase activity of the serum of two cancer susceptible strains (C3H and A) was higher than that of an insusceptible strain (C57). The esterase content of liver and the excretion of the enzyme were therefore studied. It was found that the livers of these strains were equally potent as regards esterase activity. Excretion of the enzyme, however, was very different in the two strains, being much greater in the insusceptible one. The values expressed in terms of cubic centimeters of decinormal NaOH per gram of the dry tissue were 52.96 cc. for the insusceptible strain, and 17.14 cc. and 17.49 cc. for the C3H and A strains, respectively. No difference appeared in kidney esterase activity. The findings suggest that the difference between the susceptible and insusceptible strains is not in the capacity of the liver to manufacture the enzyme but, probably, in its ability to maintain equivalent levels in the blood. A probable influence of liver metabolism on malignant growth is discussed. —Authors' abstract.

DICKENS, F., and WEIL-MALHERBE, H. [Royal Victoria Infirmary, Newcastle upon Tyne, England] THE METABOLISM OF NORMAL AND TUMOR TISSUE. XX. A COMPARISON OF THE METABOLISM OF TUMORS OF LIVER AND SKIN WITH THAT OF THE TISSUE OF ORIGIN. Cancer Research, 3:73-87. 1943.

The metabolism of tumors of the liver and skin has been studied in relation to that of their tissue of origin.

Two types of liver tumor have been used: (a) malignant liver tumors induced by feeding *p*-dimethylaminoazobenzene to rats; (b) highly differentiated spontaneous hepatomas of mice.

The skin tumors studied were carcinomas of the human vulva, and these were compared with the intermediate leukoplakia and normal skin of the vulva.

The respiration, glycolysis, and respiratory quotient of malignant liver tumors were similar to those found generally in tumor metabolism; *viz.*, high anaerobic and moderately high aerobic glycolysis combined with an R.Q. below unity. In confirmation of Orr and Stickland, it was found that in these tumors the glycogenolysis typical of normal adult liver is replaced by glucose breakdown. Precancerous states of the liver induced by *p*-dimethylaminoazobenzene, showed only a slight increase of aerobic glycolysis, and in some cases a slight anaerobic glucolysis also. The benign spontaneous mouse tumors had respiration, glycolysis, and R.Q. substantially the same as that of the adjacent liver tissue.

The following highly specialized functions were studied in both types of hepatic tumor, and were compared with the surrounding nontumorous liver tissue: formation of urea from ammonia, formation of urea from *l*(+)-alanine, formation of acetoacetic acid from caprylic acid, oxidation of uric acid, synthesis of fermentable carbohydrate from pyruvic acid.

These highly differentiated functions were entirely, or almost entirely, lost in the malignant tumors induced by *p*-dimethylaminoazobenzene, though urea synthesis usually persisted at a measurable but greatly reduced rate even after the other reactions were lacking. On the other hand, the spontaneous mouse hepatomas still retained almost intact all the foregoing functions of the normal liver cell.

The respiration and glycolysis of carcinoma of the vulva were typical of those of malignant growths in general, both aerobic and anaerobic glycolysis being high. On the other hand, the leukoplakia showed only a slight increase of both the latter reactions as compared with the normal skin of the vulva.

The interpretation of none of these results is affected by the use of the nucleic acid phosphorus content of the tissue, instead of its dry weight, as a basis for the calculation of the metabolic values.

The loss of special liver functions by the *p*-dimethylaminoazobenzene tumors is regarded as a reversion to a more primitive type of metabolism. It is suggested that the appearance of glucolysis, aerobically and anaerobically, in this as well as in other tumors, is a metabolic expression of the same primary process of dedifferentiation.—Authors' summary.

KLINE, B. E. [Univ. of Wisconsin, Madison, Wis.] THE EFFECT of *p*-DIMETHYLAMINOAZOBENZENE ON THE FORMATION OF BLOOD PROTEINS. Cancer Research, 3: 117-119. 1943.

The carcinogenic azo dyes show a high degree of specificity in inducing tumors of the liver and hence might be expected to affect certain liver functions. Since carcinogenesis probably involves protein synthesis, the proteins that are produced in the liver and can be measured in the blood offer a means of detecting a precarcinogenic chemical change due to the influence of the dye. Liver cancer was produced in rats by feeding synthetic diets containing *p*-dimethylaminoazobenzene. Plasma albumin, fibrinogen, and total nitrogen were measured at intervals during the carcinogenic period, and hemoglobin was determined at 6 months. There were no changes in blood proteins that could be associated with the process of carcinogenesis. When the diet contained *p*-aminobenzoic acid, cirrhosis was greatly reduced without changing the incidence of liver cancer.—Author's abstract.

MUELLER, G. C., and RUSCH, H. P. [Univ. of Wisconsin, Madison, Wis.] IRRADIATED BENZPYRENE AND INHIBITION OF UREASE ACTIVITY. Cancer Research, 3:113-116. 1943.

Benzpyrene was dissolved in aqueous solutions of caffeine and its effect on the activity of a urease system determined. Freshly prepared solutions were relatively nontoxic to the enzyme, but irradiation of the hydrocarbon solution with ultraviolet light produced at least two types of substances that definitely inhibited urease activity. One of these agents could be detected only after prolonged irradiation and presumably was derived from the benzpyrene, while the second inhibitory substance proved to be hydrogen peroxide. Apparently benzpyrene acted as a photosensitizing catalyst in the production of H_2O_2 during ultraviolet irradiation.—Authors' abstract.

SHARPLESS, G. R. [Henry Ford Hosp., Detroit, Mich.] DIET AND EPITHELIAL HYPERPLASIA IN THE FORESTOMACH OF RATS AND MICE. Cancer Research, 3:108-112. 1943.

Under the conditions described a deficiency of cystine, riboflavin, pyridoxin, or choline will induce hyperplasia and ulceration of the forestomach epithelium in rats. Sodium taurocholate or pepsin and hydrochloric acid increased the incidence of lesions in animals fed the poor diets but caused no macroscopic changes in rats fed the stock diet. Neither did limitation of intake of the stock diet cause any visible pathologic change in the forestomach, and the body weight remained constant in young rats. The action of the protective factors is interdependent so that a deficiency of one may prevent effective action by others. On the basis of these facts and the observation that squamous epithelial changes were occasionally observed in areas other than the stomach, it is postulated that the mechanism of formation of the gastric lesions is irritation of abnormally sensitive epithelium by hair, hard food particles, pepsin and hydrochloric acid, or bile.—Author's abstract.

Viruses

BERNHEIM, F., BERNHEIM, M. L. C., TAYLOR, A. R., BEARD, D., SHARP, D. G., and BEARD, J. W. [Duke Univ. School of Med., Durham, N. C.] A FACTOR IN DOMESTIC RABBIT PAPILLOMA TISSUE HYDROLYZING THE PAPILLOMA VIRUS PROTEIN. Science, 95:230-231. 1942.

Domestic rabbits were inoculated with virus protein in scarified areas on the abdomen and sides. The resulting growths were sliced off when they were 1 to 2 mm. high, washed in 0.9% NaCl solution, and ground with sand in 0.05 *M* phosphate buffer of pH 6.5. The tissue suspension was decanted, diluted, and 1.0 cc. placed in each of 2 tubes. To one tube, 1.0 cc. of a solution containing 2.0 mgm. of virus protein was added, and to the second, 1.0 cc. of water. In a third tube 1.0 cc. of buffer was added to 1.0 cc. of virus protein solution. Two-tenths cc. of 0.1 *M* NaCN and 0.1 cc. of toluene were added to all tubes, which were then incubated at 37° C.

The papilloma tissue underwent autolysis, but in the presence of the virus protein more carboxyl groups were liberated. Since the virus alone remains stable, this increase in carboxyl groups may be attributed to the hydrolysis of the virus by the papilloma tissue.

The hydrolyzing factor, presumably an enzyme, is present in papilloma tissue of the domestic rabbit but not in that of the cottontail rabbit. It also appears to be specific for the virus protein or similar compounds.—M. B.

Cytology

PALETTA, F. X., and COWDRY, E. V. [Washington Univ. Sch. of Med. and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] INFLUENCE OF COLCHICINE DURING METHYLCHOLANTHRENE EPIDERMAL CARCINOGENESIS IN MICE. Am. J. Path., 18:291-311. 1942.

Attempts were made to determine in mice the influence of colchicine upon the mitoses in embryo hair follicles, in adult hair follicles made hyperplastic with methylcholanthrene, and in squamous cell carcinoma induced with methylcholanthrene. At suitable intervals after the colchicine injection, tissues were removed and subjected to ultracentrifugation to study displacement of chromosomal clumps. Other special technics were also employed: Feulgen reaction for thymonucleic acid, mitochondrial stains, micro-incineration for mineral constituents.

The authors concluded that slight differences existed in the "susceptibility to colchicine of mitoses" in the above three categories. Clumping of chromosomes after colchicine injection was most pronounced in cancer cells, less in hyperplastic hair follicles, and least in embryonic hair follicles. In the embryo hair follicles, displacement of chromosome clumps by ultracentrifugation was slightly greater after colchicine than in control tissue; no such effect of colchicine was observed in either hyperplastic hair follicles or carcinoma cells. No definitive findings by the other technics were reported.—H. B.

Miscellaneous

BAUMBERGER, J. P., SUNTZEFF, V., and COWDRY, E. V. [Barnard Free Skin and Cancer Hosp. and Washington Univ., St. Louis, Mo.] METHODS FOR THE SEPARATION OF EPIDERMIS FROM DERMIS AND SOME PHYSIOLOGIC AND CHEMICAL PROPERTIES OF ISOLATED EPIDERMIS. J. Nat. Cancer Inst., 2:413-423. 1942.

Epidermis can be loosened from the underlying dermis by treating the skin with isotonic ammonia for 35 minutes or by submitting it to 50° C. for 2 minutes. The epidermis can then be removed as a complete sheet by blunt dissection. The epidermal cells thus treated adhere again to the dermis if not promptly separated. Epidermis isolated by either procedure continues to consume oxygen, as shown by manometric measurements.—A. C.

KAHLER, H. [Nat. Cancer Inst., Bethesda, Md.] APPARATUS FOR MILKING MICE. J. Nat. Cancer Inst., 2:457-458. 1942.

A description of an automatic milking apparatus by means of which 0.7 cc. of milk per mouse can be obtained. —R. C. R.

Clinical and Pathological Reports

Skin and Subcutaneous Tissues

MURRAY, M. R., and STOUT, A. P. [Coll. of Physicians and Surgeons and Presbyterian Hosp., New York, N. Y.] THE GLOMUS TUMOR. INVESTIGATION OF ITS DISTRIBUTION AND BEHAVIOR, AND THE IDENTITY OF ITS "EPITHELIOID" CELL. Am. J. Path., 18:183-203. 1942.

The glomus tumor is reputed to arise from the glomic structure (the highly specialized arteriovenous anastomosis), which has been found only in certain parts of the hands and feet at the cutaneous-subcutaneous junction. However, glomus tumors have been found in areas widely scattered over the surface of the body and also in deeper tissues such as striated muscle and those of the joint capsule. As a result of tissue culture studies of material from an infiltrative glomus tumor as well as from 2 benign glomus tumors, the authors have concluded that the characteristic "epithelioid" cell is the "pericyte" of Zimmermann (Ztschr. f. Anat. u. Entwicklungsgesch., **68**, 29-109. 1923) found around capillaries in numerous organs. This identification offers a satisfactory explanation of the occurrence of glomus tumors in regions where the normal glomus has not been demonstrated.

In the tissue cultures from the infiltrative glomus tumor, growing capillary buds were observed to become encrusted by "epithelioid" cells (pericytes); the latter cells were characterized by delicate branched pseudopodia and by a globular nucleus with 1 or 2 nucleoli and, commonly, with clear vacuoles. Silver impregnation methods were necessary to bring out the details of the processes completely. No myofibrils were seen in the pericytes, nor was any cell seen to contract. Morphologically the epithelioid cell resembles the pericyte as described by Zimmermann, and although it is related to the common adventitial cell of Clark and Clark (Am. J. Anat., **66**:1-49. 1940) it is thought to be a distinct type.—H. B.

RULISON, R. H. [New York, N. Y.] MALIGNANT MELANOMA. J. A. M. A., 119:1254. 1942.

Report of a case in which 9 years and 3 months intervened between the removal of a malignant melanoma of the skin and the appearance of metastatic growths.—H. G. W.

Female Genital Tract

COLLINS, C. G., BEACHAM, W., and SCHATTENBERG, H. J. [Tulane Univ. Med. Sch., New Orleans, La.] ARRHENOBLASTOMA OF THE OVARY. New Orleans, M. & S. J., 94:589-592. 1942.

The report of a case.—H. G. W.

CROSSEN, H. S. [St. Louis, Mo.] THE MENACE OF "SILENT" OVARIAN CARCINOMA. J. A. M. A., 119: 1485-1489. 1942.

In the author's opinion, the danger is great enough to warrant removal of the involuting ovaries whenever the abdomen is opened under circumstances which permit such removal. The author stresses also: insistence upon regular periodic pelvic examinations; utilization of every opportunity afforded by anesthesia for a minor vaginal operation, to make deep accurate palpation of the ovarian areas.—H. G. W.

FOX, R. A. [New York Post-Graduate Med. Sch. and Hosp., Columbia Univ., New York, N. Y.] BRENNER TUMOR OF THE OVARY. CASE REPORTS, DISCUSSION AND BIBLIOGRAPHY. Am. J. Path., 18:223-235. 1942.

The author adds 4 instances of Brenner tumor of the ovary to the 166 found in the literature. In 3 of the new cases and in 10 of those previously reported the tumors were bilateral. In the 4 cases described the tumors varied in size from tiny nodules to masses as large as a man's head. They were either entirely solid or occurred as nodules in the wall of an ovarian cyst. Microscopically they were composed of an abundant fibrillary connective tissue in which there were small solid nests of polyhedral cells with at times "cystic degeneration" in the center. The cyst-like spaces in the centers of the nests were lined by radially arranged columnar cells and contained mucoid secretion. The histogenesis of this benign and physiologically inert tumor is unknown.—H. B.

GNASSI, A. M., FAISON, J. B., and FELLMAN, M. [Medical Center, Jersey City, N. J.] GRANULOSA CELL CARCINOMA. REPORT OF 2 CASES. Am. J. Roentgenol., 47:458-462. 1942.

Two cases are reported in which rapidly fatal granulosa cell tumors with metastases occurred in women during the reproductive age. Most of these tumors are radiosensitive but not radiocurable; complete surgical removal is advised. Seven photomicrographs are presented.—C. E. D.

HARRIS, W. H. [Tulane Univ. School of Med., New Orleans, La.] GRANULOSA CELL TUMORS OF THE OVARY. Surg., Gynec. & Obst., 75:245-251. 1942.

A case is reported of granulosa cell tumor of the ovary with metastases to the terminal ileum. There was evidence of endocrine activity, as manifested by the hyperplastic proliferative endometrium.—H. G. W.

HOBBS, J. E. [Washington Univ. Sch. of Med., St. Louis, Mo.] PRIMARY CARCINOMA OF THE FALLOPIAN TUBE. South. M. J., 35:733-737. 1942.

A case report.—H. G. W.

MEZER, J. [Harvard Med. Sch., Boston, Mass.] METAPLASIA AND CARCINOMA IN CERVICAL POLYPS. Surg., Gynec. and Obst., 75:239-244. 1942.

Cervical polyps are common, being found in from 1.5 to 10% of gynecological patients. A third of the polyps studied were subject to squamous metaplasia, which is neither a precancerous nor a malignant process. Of 1,636 polyps studied, carcinoma arose in but 5.—H. G. W.

RESNISKY, A. F. [Hartford, Conn.] CARCINOMA OF THE UTERINE FUNDUS. Connecticut M. J., 6:173-174. 1942.

Dilatation and curettage, pathological examination, and careful subsequent observation should be carried out as a routine in any type of bleeding after the menopause. —R. C. R.

Male Genital Tract

ALYEA, E. P., and HENDERSON, A. F. [Duke Univ. Sch. of Med., Durham, N. C.] CARCINOMA OF THE PROSTATE. IMMEDIATE RESPONSE TO BILATERAL ORCHIECTOMY; CLINICAL AND X-RAY EVIDENCE. J. A. M. A., 120: 1099-1102. 1942.

A series of 40 cases of carcinoma of the prostate in which bilateral orchidectomy has been done shows remarkable im-

mediate favorable results. Pronounced subjective improvement is associated with lowering of the serum acid phosphatase, x-ray signs of healing of bone metastases, and the disappearance of pulmonary metastases. Diethylstilbestrol causes a similar improvement but not in the same degree. No prophecy is made as to what the results will be after the first postoperative year.—H. G. W.

BELT, E. [New York, N. Y.] RADICAL PERINEAL PROSTATECTOMY IN EARLY CARCINOMA OF THE PROSTATE. J. Urol., 48:287-297. 1942.

Of 50 patients operated upon by radical perineal prostatectomy, 58% have not shown a return of the disease for various periods, 24% having been free of the disease for over 4 years.—H. G. W.

CAMPBELL, H. E. [Harvard Med. Sch., Boston, Mass.] INCIDENCE OF MALIGNANT GROWTH OF THE UNDESCENDED TESTICLE. A CRITICAL AND STATISTICAL STUDY. Arch. Surg., 44:353-369. 1942.

It has been the prevalent belief heretofore that the abdominal testicle is less liable than the inguinal to malignant change. This gave rise to the practice of putting the inguinal testicle back into the abdomen if it could not be conveniently returned to the scrotum. In a critical analysis of the data reported the author concludes that the undescended testicle is more susceptible to malignant change than is the scrotal, and that the abdominal is more prone to malignant disease than the inguinal testis. The ratio of malignant tumors in the inguinal testis to those in the abdominal testis is 1 to 4. Thus one in about 20 abdominal testicles shows malignant change as compared with one in about 80 inguinal.—R. C. R.

CREEVY, C. D. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] THE DIAGNOSIS AND TREATMENT OF EARLY CARCINOMA OF THE PROSTATE. J. A. M. A., 120:1102-1105. 1942.

Radical perineal prostatectomy alone offers any real hope of cure, although its rate of applicability is low. The role of endocrine preparations in the therapy of the disease in its early stages offers interesting possibilities.—H. G. W.

DOCKERTY, M. B., and PRIESTLEY, J. T. [Mayo Clinic, Rochester, Minn.] DERMOID CYSTS OF THE TESTIS. J. Urol., 48:392-400. 1942.

Only 3 dermoids were found in a series of about 400 testicular tumors surgically removed at the Mayo Clinic. —H. G. W.

EVANS, N., BARNES, R. W., and BROWN, A. F. [Los Angeles County Hosp., Los Angeles, Calif.] CARCINOMA OF THE PROSTATE. CORRELATION BETWEEN THE HISTOLOGIC OBSERVATIONS AND THE CLINICAL COURSE. Arch. Path., 34:473-483. 1942.

From a study of 100 cases, it is concluded that a histologic grade of carcinoma of the prostate based on the degree of abnormality of the acini, the cells, and the nuclei, is of use in predicting the length of survival of the patient. The grade of the carcinoma indicates the probability of the occurrence of metastasis. The higher grades of carcinoma occur slightly earlier in life than do the lower. Roentgen therapy appears to lengthen survival time, especially in patients with the higher grades of carcinoma.—H. G. W.

GUTMAN, A. B. [Coll. of Physicians and Surgeons and Presbyterian Hosp., New York, N. Y.] SERUM "ACID" PHOSPHATASE IN PATIENTS WITH CARCINOMA OF THE PROSTATE GLAND. PRESENT STATUS. J. A. M. A., 120: 1112-1116. 1942.

Acid phosphatase is elaborated by the acinar epithelium of the prostate gland, but its function is not yet known. Carcinomatous prostate tissue contains large amounts of acid phosphatase. This may have some causal relation to the common osteoplastic character of the bone metastases, and accounts for the increased amount demonstrable in the blood in most cases of prostatic carcinoma. Elevated values for acid phosphatase were obtained in about 85% of a total of 177 cases of proved or suspected metastatic prostate carcinoma, while about 90% of patients with prostatic carcinoma without x-ray evidence of bone metastases gave figures within normal limits. Serum alkaline phosphatase also is usually increased after metastatic spread of the carcinoma and depends on the osteoplastic reaction of bone at the site of the osseous metastases. In cases of carcinoma of the prostate in which castration is performed there is usually a sharp fall in the serum acid phosphatase. The determination of serum acid phosphatase is of great value in differentiating other carcinomas with bone metastases, and Paget's disease of bone. It is useful also in detecting the presence of metastases before they are recognizable by x-ray examination.—H. G. W.

HAMM, F. C. [Brooklyn Hosp., Brooklyn, N. Y.] CLINICAL ASPECTS OF CARCINOMA OF THE PROSTATE. REVIEW OF 38 OPERATIVE CASES. J. Urol., 48:174-186. 1942.

A clinical study.—H. G. W.

HECKEL, N. J., and KRETSCHMER, H. L. [Presbyterian Hosp. and Univ. of Illinois, Chicago, Ill.] CARCINOMA OF THE PROSTATE TREATED WITH DIETHYLSTILBESTEROL. HISTOLOGIC ALTERATIONS. J. A. M. A., 119:1087. 1942.

Pronounced hydropic degeneration and vacuolation of the neoplastic cells were shown by biopsy.—H. G. W.

HERBST, W. P. [Georgetown Univ. Med. Sch., Washington, D. C.] BIOCHEMICAL THERAPEUSIS IN CARCINOMA OF THE PROSTATE GLAND. PRELIMINARY REPORT. J. A. M. A., 120:1116-1120. 1942.

The biochemical control of cancer of the prostate is based on the maintenance of a low androgen and acid phosphatase blood level. Control has been maintained for as long as 22 months by the use of estradiol dipropionate and for as long as 2½ years or more by castration. Chute advocates castration and large doses of diethylstilbestrol. Testosterone elevates blood phosphatase and therefore should be administered with caution. The dose of estrogens to be administered should not be more than that required to maintain control of the malignant process. In some instances, the malignant process may be accelerated by diethylstilbestrol. Should the cancer cease to remain quiescent in the castrated patient, as carcinoma of the breast sometimes shows renewed activity after a quiescent period following bilateral oophorectomy, it would seem likely that control may be regained by giving estradiol dipropionate or diethylstilbestrol and possibly other biochemical agents not yet utilized or available.—H. G. W.

KAHLE, P. J., OGDEN, H. D., Jr., and GETZOFF, P. L. [Sch. of Med., Louisiana State Univ., New Orleans, La.] THE EFFECT OF DIETHYLSTILBESTROL AND DIETHYLSTILBESTROL DIPROPIONATE ON CARCINOMA OF THE PROSTATE GLAND. I. CLINICAL OBSERVATIONS. J. Urol., 48:83-98. 1942.

Having observed that diethylstilbestrol and diethylstilbestrol dipropionate caused retrogression of the epithelium when administered to patients with hypertrophy of the prostate, the authors used these substances in 7 cases of carcinoma of this organ. In all patients treated there was prompt relief from pain and urinary symptoms, a general improvement in health, and a regression of the malignant lesion. The prostate glands became so altered that a diagnosis of carcinoma would have been impossible by rectal palpation. There was a regression of metastases in the lymph nodes in the 2 cases in which metastases were present, and a regression of metastatic lesions in bones in the only instance in which serial roentgenologic observations were possible. These regressions could be readily correlated with the pronounced regressive tissue changes observed in the histologic study of specimens secured by biopsy after treatment.—H. G. W.

NESBIT, R. M., and CUMMINGS, R. H. [Univ. of Michigan Med. Sch., Ann Arbor, Mich.] PROSTATIC CARCINOMA TREATED BY ORCHIECTOMY. A PRELIMINARY REPORT BASED ON SEVENTY-FIVE CASES OBSERVED FOR AT LEAST SIX MONTHS FOLLOWING OPERATION. J. A. M. A., 120:1109-1111. 1942.

Seventy-five cases of prostatic cancer treated by orchidectomy have been observed over periods of at least 6 months. Preoperative elevation in the serum acid phosphatase level was found in 39% of the cases in which the determination was made. Favorable response as determined by both subjective and objective criteria has been observed in 73% of the cases. There have been 20 failures, 10 of which were delayed with postoperative relapse at periods of from 3 to 22 months. This fact offers disquieting implications regarding the ultimate outcome in any instance.—H. G. W.

PRINCE, C. L. [Univ. of Virginia Sch. of Med., Charlottesville, Va.] MALIGNANT TUMORS OF THE SPERMATIC CORD: A BRIEF REVIEW WITH PRESENTATION OF A CASE OF ANGIO-ENDOTHELIOMA. J. Urol., 47:793-799. 1942.

A case of angio-endothelioma of the spermatic cord, the first tumor of this type reported, is added to the 74 cases of malignant tumor of the cord found in the literature.—H. G. W.

PRINCE, C. L. [Univ. of Virginia Med. Sch., Charlottesville, Va.] RHABDOMYOSARCOMA OF THE TESTICLE. J. Urol., 48:187-195. 1942.

Two new cases are added to the 15 reported in the literature. The tumor in one of the new cases weighed 2,050 gm. The histogenesis and structure of rhabdomyosarcomas of the testicle are discussed in some detail and the clinical features of the previously reported cases are reviewed.—H. G. W.

RAVICH, A. [Jewish Hosp., Brooklyn, N. Y.] THE RELATIONSHIP OF CIRCUMCISION TO CANCER OF THE PROSTATE. J. Urol., 48:298-299. 1942.

A survey of over 840 persons requiring prostatic operation reveals 1.7% of cancer among 768 Jewish patients as against 20% of cancer among 75 non-Jews.—H. G. W.

SCHENKEN, J. R., BURNS, E. L., and KAHLE, P. J. [Sch. of Med., Louisiana State Univ., New Orleans, La.] THE EFFECT OF DIETHYLSTILBESTROL AND DIETHYLSTILBESTROL DIPROPIONATE ON CARCINOMA OF THE PROSTATE GLAND. II. CYTOLOGIC CHANGES FOLLOWING TREATMENT. J. Urol., 48:99-112. 1942.

Histologic studies were made on tissues from 6 patients with carcinoma of the prostate who were being treated with diethylstilbestrol and diethylstilbestrol dipropionate. In all 6, definite retrogressive changes in the nucleus and cytoplasm of the tumor cells were observed. The ultimate change that may occur in these neoplastic cells is undetermined.—H. G. W.

SULLIVAN, T. J., GUTMAN, E. B., and GUTMAN, A. B. [Coll. of Physicians and Surgeons and Presbyterian Hosp., New York, N. Y.] THEORY AND APPLICATION OF THE SERUM "ACID" PHOSPHATASE DETERMINATION IN METASTASIZING PROSTATIC CARCINOMA; EARLY EFFECTS OF CASTRATION. J. Urol., 48:426-458. 1942.

This is a study of the results of serum acid phosphatase determinations made in 200 cases of prostatic carcinoma. In 33 of the cases castration had been performed. As an indicator of the presence of metastatic carcinoma the method failed (less than 3 units per 100 cc. of serum) in approximately 15% of 130 patients with prostatic carcinoma presenting definite or suggestive roentgenographic evidence of metastases; the remaining 85% showed an increase in serum acid phosphatase, which in extreme cases exceeded 1,000 units per 100 cc. of serum. Approximately 89% of 70 patients with prostatic carcinoma but showing no roentgenographic evidence of skeletal metastases gave values of less than 3 units per 100 cc. as did all 85 patients with diseases of the prostate gland other than carcinoma. Of a control group of 570 patients with nonprostatic disease, 90% had serum acid phosphatase levels of less than 3 units per 100 cc.; 7.5% gave values between 3 and 4.9 units per 100 cc.; and 2.5% gave values of more than 5 units per 100 cc. (maximum 9.0 units per 100 cc.). The conclusion drawn from these observations is that the method is consistent and specific enough to be of value though not an infallible supplement to clinical, roentgenographic, and other procedures for the diagnosis of metastasizing prostatic carcinoma. Castration in 31 patients with metastasizing prostatic carcinoma and serum acid phosphatase levels ranging from 520 to 4.2 units per 100 cc. resulted in an early precipitous fall in serum acid phosphatase until, after 2 or 3 months, an equilibrium was reached. The effect of castration on the serum alkaline phosphatase was more variable but eventually, after many months, equilibrium was reached. Most of the patients showed remarkable clinical improvement following castration.—H. G. W.

THOMPSON, G. J. [Mayo Clinic, Rochester, Minn.] TRANSURETHRAL RESECTION OF MALIGNANT LESIONS OF THE PROSTATE GLAND. J. A. M. A., 120:1105-1109. 1942.

In 95% of cases the disease has spread beyond the confines of the prostate gland when the patient is first observed, so that in but 5% or less radical resection is justifiable. Transurethral resection is the most desirable operation for the relief of obstruction; combined with bilateral orchidectomy and the administration of diethylstilbestrol, it offers the best method of treatment now available for patients who have retention of urine.—H. G. W.

Gastrointestinal Tract

GOLDMAN, C., and ROBILLARD, G. L. [Cumberland Hosp., Brooklyn, N. Y.] MALIGNANT MELANOMA OF THE RECTUM. REPORT OF A CASE. Am. J. Surg., 57:352-355. 1942.

Melanomas of the intestinal tract constitute 2 to 3% of all melanomas and are most frequently found in the rectum. A case is reported.—H. G. W.

JUDD, E. S., Jr. [Mayo Clinic, Rochester, Minn.] RESIDUAL LESIONS OF ULCERATIVE GASTRITIS. POSSIBLE RELATIONSHIP TO THE DEVELOPMENT OF CARCINOMA OF THE STOMACH. Surg., Gynec. and Obst., 75:424-432. 1942.

A microscopic study was made of sections from 200 carcinomatous and 78 control stomachs, and the occurrence of certain residual lesions frequent in carcinomatous stomachs is compared to their presence in the noncarcinomatous organ. Evidence is presented to indicate that essentially similar lesions occurring at a distance from a gastric carcinoma suggest that the entire gastric mucosa has undergone a change and that much time has been required for this change to take place. Carcinoma develops in a previously damaged stomach and many years of such injury may be required before neoplastic transformation begins. The pathogenesis of gastric carcinoma is directly related to the disorganized hyperplasia of gastric mucous cells.—H. G. W.

KAHN, M., and BAY, M. W. [Univ. of Southern California Med. Sch., Los Angeles, Calif.] CARCINOMA OF THE JEJUNUM. Am. J. Surg., 58:145-147. 1942.

Two cases are reported, with autopsy and surgical statistics.—H. G. W.

MORTON, C. B. II [Univ. of Virginia, Charlottesville, Va.] TOTAL GASTRECTOMY. INDICATIONS FOR OPERATION WITH A REPORT OF FOUR CASES. Arch. Surg., 44: 72-80. 1942.

The anatomical relationships of the stomach allow the spread of tumor cells to adjacent areas with relative ease, and after radical subtotal gastric resection a microscopically demonstrable tumor often remains. In most cases of subtotal gastric resection the operation of choice would be total resection if the procedure were not complicated by so many technical difficulties. These, together with a general ignorance of the physiological implications of total gastric resection cause a high operative mortality and thus militate against the more radical procedure. It is thought, however, that more total resections should be done, together with widespread lymphatic removal, and 4 cases are reported in defense of this thesis. An operative technic is described.—R. C. R.

RUBIN, J. S. [Jamaica, N. Y.] PROLAPSE OF POLYPOID GASTRIC MUCOSA INTO THE DUODENUM, WITH MALIGNANT CHANGE. Radiology, 38:362-364. 1942.

A case is reported of a patient who had suffered symptoms suggestive of a partial pyloric obstruction for over a year. Roentgenograms are reproduced which led to the diagnosis of "polypoid herniation of gastric antral mucosa into duodenal bulb." Herniation was confirmed at operation. Histological study revealed polyposis of the stomach with malignant degeneration.—C. E. D.

SCHINDLER, R., and ARNDALE, O., (University of Chicago, Chicago, Ill., and Glendale, Calif.) GASTROSCOPIC DIFFERENTIAL DIAGNOSIS OF BENIGN AND MALIGNANT ULCER OF THE STOMACH. AN ANALYSIS OF THE GASTROSCOPIC PICTURE OF ONE HUNDRED AND THIRTY-THREE LESIONS. Arch. Surg. 44:473-488. 1942.

Gastroscopy is the best mode of differentiating benign and malignant ulcers of the stomach. In comparing diagnoses by gastroscopic and roentgen examination it was found that gastroscopy missed more lesions, but a noncommittal diagnosis was made in only 2 cases, whereas roentgen examination made no commitment in 15.

Gastroscopic signs favoring benign ulcers, signs that are not proof of benign ulcer, those that are not proof of malignant ulceration, and those that suggest malignant ulcer are discussed. It is admitted that in 6 of 113 cases a correct diagnosis could not be made. The simplicity, ease, and obvious value of the procedure make gastroscopy an important diagnostic adjunct.—R. C. R.

SCHINDLER, R., and LETENDRE, P. [Univ. of Chicago, Chicago, Ill.] ANALYSIS OF RELATIONSHIP OF SURGERY AND GASTROSCOPY IN 95 CASES OF GASTRIC TUMOR. Surg., Gynec. & Obst., 75:547-557. 1942.

In 5 of 91 cases of gastric cancer the lesion was not detected with the gastroscope, and in 5 others the lesions seen were wrongly interpreted.—H. G. W.

SCHINDLER, R., SANDWEISS, D. J., and MINTZ, L. L. [Univ. of Chicago, Chicago, Ill.] BENIGN SUBMUCOSAL TUMORS OF THE STOMACH: A GASTROSCOPIC STUDY. Am. J. Digest. Dis., 9:289-292. 1942.

The diagnosis of submucosal benign gastric tumors is possible at gastroscopy by observation of the symptom of "bridging folds."—H. G. W.

SCHINDLER, R., and SMITH, W. M. [Univ. of Chicago, Chicago, Ill.] ANACIDITY AND GASTRITIS ASSOCIATED WITH GASTRIC CARCINOMA. Am. J. Digest. Dis., 9:340-342. 1942.

In 48 cases of gastric carcinoma the uninvaded mucosa was studied gastroscopically, and the diagnosis checked by microscopic examination. Although chronic gastritis was found definitely in 38 instances, there were 4 in which a normal mucosa was found associated with anacidity. These 4 cases indicate that chronic gastritis is not always the cause of anacidity in a stomach invaded by carcinoma.—H. G. W.

SHEARBURN, E. W. [Univ. of Virginia Hosp., Charlottesville, Va.] IMPORTANCE OF RECTAL EXAMINATION IN THE PROGNOSIS OF RECTAL CARCINOMA. J. A. M. A., 119:1410-1412. 1942.

In only 36% of a series of 100 cases referred for treatment during the past ten years has an adequate examination been made by the local physician.—H. G. W.

TUTA, J. A., and ROSI, P. A., [Univ. of Illinois Coll. of Med. and Northwestern Univ. Med. Sch., Chicago, Ill.] LYMPHOSARCOMA OF THE RECTUM. Arch. Surg., 44: 157-163, 1942.

A report of a case with a discussion of histologic features and treatment.—R. C. R.

WESTON, S. [Beth Moses Hosp., Brooklyn, N. Y.] METASTASES TO THE HUMERUS FROM CARCINOMA OF THE RECTUM. Am. J. Surg., 57:531-535. 1942.

This article places on record the sixth reported case. —H. G. W.

Leukemia, Lymphosarcoma, Hodgkin's Disease

LÖVGREN, O., and WESTMAN, C. [Central Hosp., Stocksund, Sweden] ON THE ETIOLOGY OF AND THE SUPPOSED RELATIONS BETWEEN LYMPHOGRANULOMATOSIS MALIGNA AND MYCOSIS FUNGOIDES. Acta Med. Scandinav., 108:387-397. 1941.

The difficulty in distinguishing Hodgkin's disease, clinically and pathologically, from mycosis fungoides is illustrated by 3 cases. The patients had skin eruptions considered to be characteristic of the latter disease, but microscopic examination of enlarged lymph nodes disclosed typical signs of lymphogranulomatosis. A description is appended of a case of generalized lymphadenopathy without cutaneous manifestations, which proved microscopically to be reticulum cell sarcoma.—M. J. E.

REED, E. B. [Lincoln, Neb.] SOME CLINICAL ASPECTS OF LEUKEMIA. Nebraska State M. J., 26:429-433. 1941.

A general review based upon the observation of 40 cases.—M. J. E.

SLAUGHTER, D. P., and CRAVER, L. F. [Memorial Hosp., New York, N. Y.] HODGKIN'S DISEASE. FIVE YEAR SURVIVAL RATE; VALUE OF EARLY SURGICAL TREATMENT; NOTES ON FOUR CASES OF LONG DURATION. Am. J. Roentgenol., 47:596-606. 1942.

Two hundred and sixty-five cases of histologically confirmed Hodgkin's disease are reviewed. The average survival time from the beginning of therapy was 33.8 months. Of the patients 17.7% survived 5 years and 3.4% 10 years or more. Sixty-two per cent were males and 38% females. There was no correlation between the histopathology of the tumor and the course of the disease. As a rule treatment is limited to palliative radiation and general measures. When a single group of accessible lymph nodes is involved, "obliterative" radiation or surgical extirpation is warranted.—C. E. D.

Cancer Control and Public Health

MAXFIELD, J. R. [Lovelace Clinic, Albuquerque, N. Mex.] PRESENT CONCEPT OF THE CANCER PROBLEM. Southwestern Med., 25:4-9. 1941.

This paper presents a brief review of the cancer problem and discusses the etiology, diagnosis, and treatment of the disease. A plea is made for education of the laity, for the referring of patients to specialists by the general practitioner, and for the dissemination of knowledge by cancer specialists to the other members of the profession.—J. L. M.

OHLMACHER, J. C. [Vermillion, S. Dak.] THE MEDICAL PROFESSION'S RESPONSIBILITY IN THE CONTROL OF CANCER. Journal-Lancet, 61:441-445. 1941.

A general discussion of the problems of early diagnosis and treatment.—M. J. E.

SKINNER, E. H. [Kansas City, Mo.] THE PHILOSOPHICAL AND PRACTICAL ASPECTS OF THE ECONOMICS OF CANCER CONTROL. Am. J. Roentgenol., 47:1-10. 1942.

This Janeway Lecture discusses the responsibility of America as the scientific repository of the world at war and cites the objections to substituting governmental responsibility for individual initiative and enthusiasm. Cancer control programs succeed or fail in proportion to the contagious ambition and drive of the director.

Cooperation of the general practitioner and an educated public in striving for the early diagnosis of cancer is the first necessity in effective control. Specialized consultation should be made available to country practitioners through periodic rural clinics attended by competent urban specialists. Such decentralization of specialists would be valuable in cardiology, tuberculosis, psychiatry, and orthopedics, as well as in cancer.—C. E. D.

WETHERELL, F. S. [Syracuse, N. Y.] ROLE OF THE GENERAL PRACTITIONER IN THE CURE OF CANCER. Southwestern Med., 25:67-70. 1941.

This editorial emphasizes the importance of a thorough examination of the patient, the type of information patients should be given, and the educational functions of cancer control bureaus.—J. L. M.

ORGANISATION FOR THE TREATMENT OF CANCER. National Radium Commission, London, England.

Introduction

The Registrar General's Report shows cancer to be the second most common cause of death in England and Wales, accounting for 68,605 of 478,829 deaths in 1938.

The Commission has been impressed by the fact that the death rate continues to rise, although the centers with which the Commission deals treat a constantly increasing number of cases with a slow but persistent improvement in the number of patients who survive several years after treatment. There are some remediable factors which contribute to this state of affairs, and among them are:

1. Late diagnosis, which is due to: (a) unsatisfactory conditions under which many patients are seen for the first time, *e.g.* ill-equipped consulting rooms and inadequate facilities for examination and note-taking; (b) inexperience of cancer on the part of some medical men who see only a few cases a year; (c) ignorance and fear of the disease among the laity.

2. Scarcity of beds.

3. Failure to select the most appropriate treatment, owing to the absence, in most hospitals, of any organized system for consultation, before treatment is started, between the various specialists.

4. Inefficient treatment in inadequately equipped centers, due to the natural ambition of every hospital to carry out all kinds of treatment.

Suggestions for a Cancer Organization on a Regional Basis

The functions of a cancer organization are:

1. To put before the public those facts about cancer which ought to be generally known.

2. To secure the diagnosis of cancer at the earliest possible stage.

3. To secure prompt and adequate treatment of every case.

4. To improve established methods of treatment and devise new ones.

The area to be served by a cancer organization should be of such a size that not less than 1,000 cancer cases are

treated in a year. Such a number can be expected from a population of about a million, and this should normally be the smallest number of people for which a cancer organization should be established. This number is necessary so that: (1) each member of the medical team shall see a large enough number of patients to become expert; (2) the available radium and x-ray apparatus shall be used to capacity; (3) each type and site of growth may form a group large enough for statistical analysis in a few years.

A whole time director of the cancer organization should be appointed, although it might be advisable to allow him private patients within his own hospital, but not outside it. It is important that his position at the headquarters hospital should be assured, and he should be able to meet the members of the honorary staff on equal terms and be in full and effective control of the arrangements made for carrying out the treatment of cancer.

The staff of a cancer organization, besides the whole time director, must include physicians, surgeons, E.N.T. surgeons, gynecologists, radiotherapists, pathologists, radiodiagnosticians, and physicists, who should meet in frequent consultation. Each specialist must deal with enough cases to ensure that he becomes expert.

The housing of a cancer organization, in view of the staff requirements mentioned above, will almost certainly have to be in, or in close association with, one or more large general hospitals where a special block or department should be devoted to the work. The segregation of the cancer work makes the team more "cancer minded."

Arrangements for the Diagnosis of Cancer

General medical practitioners will have to be persuaded to send promptly to the cancer organization all patients in whom any symptom calls for investigation. Treatment of cancer in any patient should be undertaken only after consultation between members of the team. The treatment, having been settled at consultation, is then carried out by one of the specialists of the team, and at appropriate intervals or at the end of the treatment the patient is again seen in consultation. The treatment of cancer by surgery might be carried out at several of the hospitals taking part in the cancer organization, but it is essential that it should be done only by the surgeons of the team (who have special experience of the operations needed) after consultation with the radiotherapist.

The Treatment of Cancer by Radiotherapy

The radiotherapist should be in full charge of all radium and x-ray therapy of the organization, although it will be necessary for him at times to work with the help of the surgeons. The pathologist must be familiar not only with all forms of cancer, but with the habits of mind and methods of work of all those engaged in the cancer organization and should be in personal contact with all members of the team. Radiodiagnosis is necessary not only for the diagnosis but in the treatment and subsequent examination of the patient; hence there must be an efficient radiodiagnostician with modern apparatus. A physicist is essential in any radiotherapy clinic and should be allowed facilities for research and be provided with a well equipped laboratory and workshop and a whole time mechanic. The setting of every patient each time he is treated should be done by one of the radiotherapists or under his direct supervision, but the physicist will frequently be needed to aid in beam direction and to see that patient and staff are adequately protected from stray radiation.

The beds for the cancer organization should be in a special block in which the x-ray therapy plant is situated and radium treatment carried out. Not less than 75 beds will be needed for the cancer organization.

The Follow-Up of Cancer Patients

The patient should attend at regular intervals and be seen by those members of the team who originally planned the treatment. All cancer patients should be followed up for at least 5 years, though much may be learnt by continuing to examine them for 10 years, or even longer.

The records of a cancer organization must provide a basis for statistical analysis of the results obtained by treatment; there should be a special case paper for the cancer organization, which should be independent of the ordinary hospital in-patient case paper.—E. L. K.

Correction

See: Proceedings of the American Association for Cancer Research, Inc., 35th Annual Meeting. Cancer Research, **3**:120-139. 1943.

P. 125. Abstract entitled "The Distribution and Prevalence of a Carcinogenic Factor in Human Livers" by Paul E. Steiner. This paper is not the one published in Vol. 2, pages 425-435, as stated in the Proceedings. It is, however, to be published in Cancer Research, Vol. 3, 1943, with the title "The Incidence of a Carcinogenic Factor in the Livers of Cancer, Noncancer, Cirrhotic, and Negro Patients."